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Plate Incorporation Mutagenicity Study of
Whole Smoke Condensate of Cigarettes
LEAR 0-17-2, LEAR 0-17-3, LEAR 2-17-2,
LEAR 2-17-3, LEAR 4-17-2 and LEAR 4-17-3
and of Standard Reference Cigarette 2R1
on *Salmonella* *Typhimurium* Strains TA 98 and TA 100

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This report, including front page, contains 153 pages.

ABBREVIATIONS (a,b)

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| | |
|------------|--|
| A | : absorbance |
| 2-AA | : 2-aminoanthracene |
| 2-AF | : 2-aminofluorene |
| AHM | : aryl hydrocarbon monooxygenase (EC 1.14.14.2) |
| approx. | : approximately |
| ART. | : article |
| B(a)P | : benzo(a)pyrene |
| BSA | : bovine serum albumin |
| BW | : body weight |
| CA | : cellulose acetate |
| CFU | : colony forming units |
| cig. | : cigarette |
| COEFF. | : coefficient |
| CONC. | : concentration |
| cond. | : condensate |
| CORREL. | : correlation |
| DIL. SUSP. | : diluted suspension |
| DIN | : Publication of the German Committee of Standards |
| DMSO | : dimethylsulfoxide |
| DPM | : dry particulate matter |
| EC | : enzyme code according to the "International Union of Biochemistry Commission on Enzymes" |
| equiv. | : equivalent |
| FID | : flame ionisation detector |
| G6P | : glucose-6-phosphate |
| G6PDH | : glucose-6-phosphate dehydrogenase (EC 1.1.1.49) |
| .GT. | : greater than |

(a) in addition to those, which are explained immediately on the same page in the text, tables or figures
(b) Units are given in accordance with SI-norms (Système International d'Unités).

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ABBREVIATIONS (continued)

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HPLC : high performance liquid chromatography
INDIV. : individual
ISH : SH-index, thiol reactivity of cigarette smoke
L : light
LD : light/dark
.LT. : less than
MC : moisture content
MMS : methyl methanesulfonate
MNNG : N-methyl-N'-nitro-N-nitrosoguanidine
N : number of individual values
NAD : nicotine adenine dinucleotide
NADP : nicotine adenine dinucleotide phosphate
2-NF : 2-nitroflurene
no., No. : number
OW : organ weight
pH : negative decadic logarithm of hydrogen-ion concentration
prot. : protein
REGRESS. : regression
rev. : revertants
rpm : revolutions per minute
RSD : standard deviation relative to the mean in percent
RT : room temperature
RTD : resistance to draw
SE : standard error
S9 : supernatant of 9000 x g centrifugation
SPEC. : specific
SPF : specified pathogen free
TAR : TPM minus nicotine and water
TPM : total particulate matter
Tris-HCl : tris(hydroxymethyl)aminomethane hydrochloride

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ABBREVIATIONS (continued)

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U : unit
UV : ultra-violet
WSC-I : whole smoke condensate(s) obtained by impaction trap
WW : wet weight
 $\times g$: centrifugal force in terms of the constant of gravitation
($1 \times g = 9.81 \text{ m/s}^2$)
1oEx : x is the exponent to the base of 10

o : no response
+ : response
- : not assayed

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1 SUMMARY

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1.1 Objective

The mutagenicity of whole smoke condensate of cigarettes LEAR 0-17-2, LEAR 0-17-3, LEAR 2-17-2, LEAR 2-17-3, LEAR 4-17-2 and LEAR 4-17-3 are compared in the plate incorporation mutagenicity assay with *Salmonella typhimurium* (Ames et al., 1975). The cigarette 2R1 is used as reference.

1.2 Cigarettes

LEAR 0-17-2, LEAR 0-17-3, LEAR 2-17-2, LEAR 2-17-3, LEAR 4-17-2 and LEAR 4-17-3 are filter cigarettes belonging to project "LEAR".

The cigarette 2R1 is the internationally used standard reference cigarette produced for and used by the University of Kentucky Research Foundation.

1.3 Experimental

The experiment was performed as 2 independent assays using in each *Salmonella typhimurium* TA 98 and TA 100 as tester strains. Homogenate (S9 protein) from Aroclor 1254-induced rat liver was used for metabolic activation.

The tester strains TA 98 and TA 100 are used to detect frameshift mutagens and mutagens causing base-pair substitutions, respectively. The strains were checked and found to be in accordance with the requested characteristics.

Each condensate was assayed at doses of 0, 0.05, 0.10 and 0.15 milligrams dry condensate per plate.

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The number of revertants was used to calculate the dose-response relationship. The specific mutagenicity, defined as the regression coefficient "a" of the linear dose-response curve $y = ax + b$, was calculated as the extrapolated increase in the number of revertants per milligram of dry condensate.

1.4 Results

1.4.1 Tester strain TA 98, frameshift mutation

The specific mutagenicity in assay 1 was statistically not different to that of assay 2 for the 7 cigarettes. The mean specific and relative specific mutagenicity of both assays were:

| CIGARETTE | SPECIFIC MUTAGENICITY (rev./mg) | RELATIVE SPEC. MUTAGENICITY (o/o) |
|-------------|---------------------------------------|---|
| 2R1 | 1536 | 100 |
| LEAR 0-17-2 | 2235 | 146 |
| LEAR 0-17-3 | 3508 | 228 |
| LEAR 2-17-2 | 1930 | 126 |
| LEAR 2-17-3 | 2942 | 192 |
| LEAR 4-17-2 | 2023 | 132 |
| LEAR 4-17-3 | 2988 | 195 |

The condensate of cigarettes with the last digit number "2" were found to be statistically significantly less mutagenic than the corresponding cigarettes with the last digit number "3". The cigarettes of project "LEAR" were statistically significantly higher mutagenic than 2R1.

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1.4.2 Tester strain TA 100, base-pair mutation

The specific mutagenicity in assay 1 was statistically not different to that of assay 2 for the 7 cigarettes. The mean specific and relative specific mutagenicity of both assays were:

| CIGARETTE | SPECIFIC MUTAGENICITY (rev./mg) | RELATIVE SPEC. MUTAGENICITY (o/o) |
|-------------|---------------------------------------|---|
| 2R1 | 696 | 100 |
| LEAR 0-17-2 | 859 | 123 |
| LEAR 0-17-3 | 1354 | 195 |
| LEAR 2-17-2 | 897 | 129 |
| LEAR 2-17-3 | 1185 | 170 |
| LEAR 4-17-2 | 863 | 124 |
| LEAR 4-17-3 | 1000 | 144 |

The condensate of cigarettes LEAR 0-17-2 and LEAR 2-17-2 were found to be statistically significantly less mutagenic, whereas the cigarette LEAR 4-17-2 was only numerically less mutagenic than the corresponding cigarettes with the last digit number "3". The cigarettes of project "LEAR" were statistically significantly higher mutagenic than 2R1.

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1.5 Conclusion

For frameshift mutation as well as for base-pair substitution the condensate of cigarettes with the last digit number "2" are less mutagenic than the corresponding cigarettes with the last digit number "3". The condensates of all cigarettes of project "LEAR" are more mutagenic than that of standard reference cigarette 2R1.

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2 RESPONSIBILITY

=====

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Chemist (Diplomchemiker)

Biometry:

H. Gugel
Mathematician (Diplommathematiker)

Quality Assurance:

E. Römer
Biologist (Diplombiologe)

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3 CIGARETTES
=====3.1 General Specifications

Test substances: whole smoke condensates of cigarettes prepared by impaction trap

Code of cigarettes

4 cigarettes from
project "LEAR":
LEAR 0-17-2
LEAR 0-17-3
LEAR 2-17-2
LEAR 2-17-3
LEAR 4-17-2
LEAR 4-17-3

1 standard reference
cigarette: 2R1

Date of receipt at INBIFO

LEAR cigarettes: 28.Oct.81

2R1: Apr.78

Source

LEAR cigarettes: FABRIQUES DE TABAC REUNIES S.A.
CH-2003 Neuchatel
Switzerland

2R1: PM, USA

Amount

LEAR cigarettes: 1000 each, except 0-17-2 (980)

2R1: - (taken from INBIFO stock)

Packing

LEAR cigarettes: 20 cigarettes/hard box

2R1: 20 cigarettes/soft box

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Storage and conditioning

Main storage

Walk-in cold room
(room R911):

approx. 4 degrees centigrade,
relative humidity uncontrolled
(2R1 only)

Conditioning

Conditioning room
(room R326):

cigarettes taken out of their
packaging and deposited in
open racks in upright position

stored for at least 4 d prior
to use at approx. 22 degrees
centigrade, approx. 60 o/o
relative humidity

Selection (a)

| Weight (g/cig) | assay 1 | assay 2 |
|----------------|--------------|--------------|
| LEAR 0-17-2: | 1.00 to 1.06 | 0.99 to 1.05 |
| LEAR 0-17-3: | 1.00 to 1.06 | 1.00 to 1.06 |
| LEAR 2-17-2: | 0.99 to 1.05 | 0.98 to 1.05 |
| LEAR 2-17-3: | 1.07 to 1.13 | 1.04 to 1.11 |
| LEAR 4-17-2: | 1.00 to 1.06 | 0.99 to 1.05 |
| LEAR 4-17-3: | 0.98 to 1.04 | 0.98 to 1.04 |
| RTD (mm H2O): | no selection | |

3.2 Supplier's Specifications

Specifications of cigarettes: see TABLE A

Physical properties and
chemical composition of
filler of cigarettes: see TABLE B

Specifications of filters
of cigarettes: see TABLE C

Smoke components per
cigarette: see TABLE D

(a) selected by personnel of Microbiology lab in order to use
cigarettes within a reasonable range of weight

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| CIGA- RETTE | SPECIFIED PARAMETERS | | | | | | |
|----------------|-------------------------|-------------------------------|-----------------------|-------------------------|---------------|--------------------------------|------------------------|
| | TOTAL CIG. WEIGHT | FILTER AND PAPER WEIGHT | TOBACCO WEIGHT (a) | TOTAL CIG. LENGTH | DIAMETER | RTD | STATIC BURNING TIME |
| | (029) (mg/cig.) | (030) (mg/cig.) | (031) (mg/cig.) | (-) (mm) | (025) (mm) | (033) (mm H ₂ O) | (-) (min/40 mm) |
| 2R1 | 1194 | 110 | 1084 | 85 | 7.96 | 81 | 13.7 |
| LEAR 0-17-2 | 1047 | 227 | 820 | 84 | 7.93 | 97, 95 | - |
| LEAR 0-17-3 | 1025 | 223 | 797 | 84 | 7.98 | 110, 12 | - |
| LEAR 2-17-2 | 1049 | 222 | 827 | 84 | 7.96 | 94, 101 | - |
| LEAR 2-17-3 | 1097 | 232 | 860 | 84 | 7.91 | 110, 126 | - |
| LEAR 4-17-2 | 1050 | 229 | 821 | 84 | 7.96 | 101, 104 | - |
| LEAR 4-17-3 | 1027 | 223 | 804 | 84 | 7.95 | 111, 124 | - |

TABLE A

SPECIFICATIONS OF CIGARETTES

specifications and method numbers (b) provided by the supplier

(a) at 0/0 FC (= moisture found)

(b) method numbers given in brackets just below parameters

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| CIGA- RETTE | FILLER | | | | |
|----------------|--------------------|--------------------|---------------------|---------------------|-------------------|
| | TOTAL ALKALOIDS | REDUCING SUGARS | NITRATE NITROGEN | AMMONIA NITROGEN | NITROGEN TOTAL |
| | (o74) | (o75) | (o76) | (o77) | (o79) |
| (o/o) | (o/o) | (o/o) | (o/o) | (o/o) | (o/o) |
| 2R1 | 1.98 | 10.5 | 0.20 | 0.09 | 2.17 |
| LEAR 0-17-2 | 1.93 | 7.1 | 0.26 | 0.33 | - |
| LEAR 0-17-3 | 2.49 | 0.0 | 0.50 | 0.51 | - |
| LEAR 2-17-2 | 1.63 | 7.9 | 0.10 | 0.22 | - |
| LEAR 2-17-3 | 1.60 | 0.6 | 0.02 | 0.16 | - |
| LEAR 4-17-2 | 1.76 | 7.5 | 0.16 | 0.27 | - |
| LEAR 4-17-3 | 2.05 | 0.0 | 0.28 | 0.27 | - |

TABLE B

PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION OF FILLER OF CIGARETTES
specifications and method numbers (a) provided by the supplier

(a) method numbers given in brackets just below parameters

2026048890

| CIGA- RETTE | FILLER | | | | | | | | | |
|----------------|----------|-------|-----------|---------|-----------|-----------------------|---------------|------------------------------|----------------------------------|----------------------------|
| | CHLORIDE | ASHES | POTASSIUM | CALCIUM | MAGNESIUM | HOT WATER SOLUBLES | TOBACCO pH | TOBACCO MOISTURE CONC. | EQUILIBRIUM MOISTURE CONC. | FILLING POWER CV (a) |
| | (112) | (113) | (114) | (115) | (371) | (305) | (111) | (071) | (122) | (120) |
| | (o/o) | (o/o) | (o/o) | (o/o) | (o/o) | (o/o) | (o/o) | (o/o) | (o/o) | (ml/10 g) |
| 2R1 | 0.66 | 14.8 | - | - | - | 59.0 | 5.5 | - | 12.6 | - |
| LEAR 0-17-2 | 0.72 | 14.0 | 4.23 | 2.78 | 0.51 | 51.8 | - | 12.6 | 10.6 | 36 |
| LEAR 0-17-3 | 0.71 | 18.5 | 4.54 | 3.71 | 0.59 | 44.35 | - | 10.8 | 9.1 | 44 |
| LEAR 2-17-2 | 0.60 | 13.8 | 3.79 | 2.53 | 0.44 | 42.26 | - | 12.5 | 11.6 | 31.1 |
| LEAR 2-17-3 | 0.73 | 19.9 | 5.09 | 3.74 | 0.45 | 32.63 | - | 12.1 | 9.7 | 42.6 |
| LEAR 4-17-2 | - | - | - | - | - | 51.39 | - | 12.8 | 11.7 | 40.7 |
| LEAR 4-17-3 | 0.58 | 16.5 | 4.25 | 3.64 | 0.52 | 38.99 | - | 12.6 | 13.5 | 45.9 |

TABLE B (continued)

PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION OF FILLER OF CIGARETTES

specifications and method numbers (b) provided by the supplier

(a) at 12 o/o MC (moisture content)

(b) method numbers given in brackets just below parameters

2026048891

| CIGARETTE | FILTER TYPE | MATERIAL | LENGTH (mm) | TIPPING PAPER LENGTH (mm) | RTD (mm H ₂ O) |
|-------------|-------------|----------|----------------|------------------------------|------------------------------|
| 2R1 | - (a) | - | - | - | - |
| LEAR o-17-2 | S | CA | 20 | 24 | 62 |
| LEAR o-17-3 | S | CA | 20 | 24 | 63 |
| LEAR 2-17-2 | S | CA | 20 | 24 | 66 |
| LEAR 2-17-3 | S | CA | 20 | 24 | 62 |
| LEAR 4-17-2 | S | CA | 20 | 24 | 66 |
| LEAR 4-17-3 | S | CA | 20 | 24 | 61 |

TABLE C

SPECIFICATIONS OF FILTERS OF CIGARETTES

specifications provided by the supplier

(a) no filter

2026048892

| CIGA- RETTE | PUFF COUNT | SMOKE COMPONENTS | | | | | | | | | |
|----------------|---------------|------------------|-----------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------|-------|
| | | TPM | WATER IN TPM | DPM | NICO- TINE | TAR | CO | NO | HCN | ALDE- HYDES | ISH |
| | | (a86) | (a83) | (a80) | (a84) | (a85) | (-) | (a81) | (a82) | (a88) | (a89) |
| | | (1/cig.) | (mg/ cig.) | (mg/ cig.) | (mg/ cig.) | (mg/ cig.) | (mg/ cig.) | (mg/ cig.) | (ug/ cig.) | (mg/ cig.) | (o/o) |
| 2R1 | 13.1 | 48.4 | 4.9 | 43.5 | 3.32 | 40.2 | 25.4 | 0.39 | 453 | 3.31 | 71 |
| LEAR 0-17-2 | 9.0 | 18.4 | 1.8 | 16.6 | - | - | 14.1 | 0.27 | 204 | 1.25 | - |
| LEAR 0-17-3 | 9.2 | 14.5 | 0.9 | 13.6 | - | - | 12.1 | 0.45 | 143 | 1.07 | - |
| LEAR 2-17-2 | 8.9 | 18.0 | 1.8 | 16.3 | - | - | 16.2 | 0.11 | 186 | 1.28 | - |
| LEAR 2-17-3 | 9.5 | 17.2 | 1.1 | 16.1 | - | - | 15.4 | 0.06 | 149 | 1.33 | - |
| LEAR 4-17-2 | 9.4 | 19.2 | 1.8 | 17.5 | - | - | 18.1 | 0.18 | 219 | 1.23 | - |
| LEAR 4-17-3 | 9.0 | 16.8 | 1.0 | 15.8 | - | - | 15.3 | 0.27 | 144 | 1.19 | - |

TABLE D

SMOKE COMPONENTS PER CIGARETTE

specifications and method numbers (b) provided by the supplier

(a) method numbers given in brackets just below parameters

2026048893

4 METHOD

4.1 Chronological Tables (see FIGURES A and B)

4.2 Condensate Preparation, Storage and Analyses

4.2.1 Preparation of whole smoke condensate by impaction trap (WSC-I)

Principle:

mechanical open-end smoking to a defined butt length in automatic negative pressure (vacuum pump) smoking machine, condensate collection in impaction trap

Time:

7 or 8 d before plate incorporation assay

Sample material and quantity: cigarettes (see 3 TEST SUBSTANCES),
30 cigarettes/condensate

Number of condensates prepared: 4 batches of each type of cigarette

Equipment

Smoking machine

Type: automatic INBIFO smoking machine

Number of machines:

1

Machine no.:

oo25

Loading of cigarettes:

manually into the cigarette holding device up to a depth of 9 + 1 mm in accordance with DIN 10240 (a)

Lighting of cigarettes:

automatically with an iodine spot lamp adjusted in a gold plated focal mirror

Ejection of cigarettes:

automatically at butt length of 32 to 28 mm for filter cigarettes and 23 mm for filterless standard reference cigarette (automatic scanning with infrared photodiode)

(a) Maschinelles Abrauchen von Zigaretten und Bestimmung des Rauchkondensats, DIN 10240, April 1978

2026048894

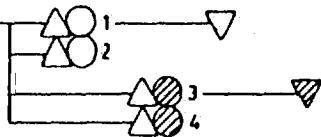
DAY

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|----|----|----|----|
|---|---|---|---|---|---|---|---|---|----|----|----|----|

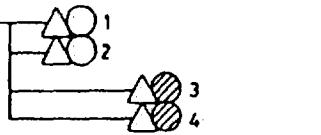
CIGA-
RETTETTE

2R1

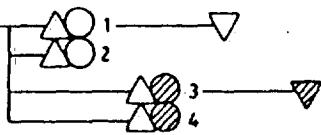
■ 54



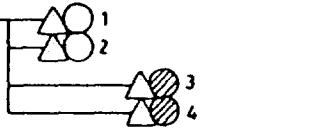
■ 55

LEAR
o-17-2

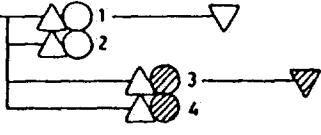
■ o1



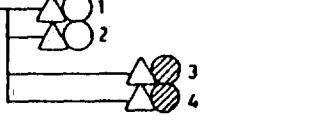
■ o2

LEAR
o-17-3

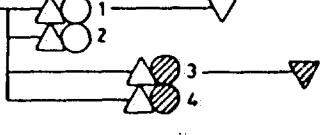
■ o1



■ o2

LEAR
2-17-2

■ o1



■ o2

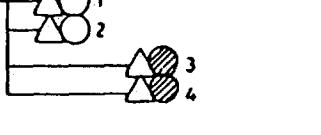


FIGURE A

CHRONOLOGICAL TABLE, ASSAY 1

WSC-I preparation (■), dilution of WSC-I suspension (△), mutagenicity assay with TA 98 (○) and with TA 100 (◎) and reversion assay with TA 98 (▽) and with TA 100 (▽) of cigarette smoke condensates. The batch numbers of WSC-I suspension and dilution of WSC-I suspensions are shown beside the corresponding symbols.

Day 1: 2.Nov.81

2026048895

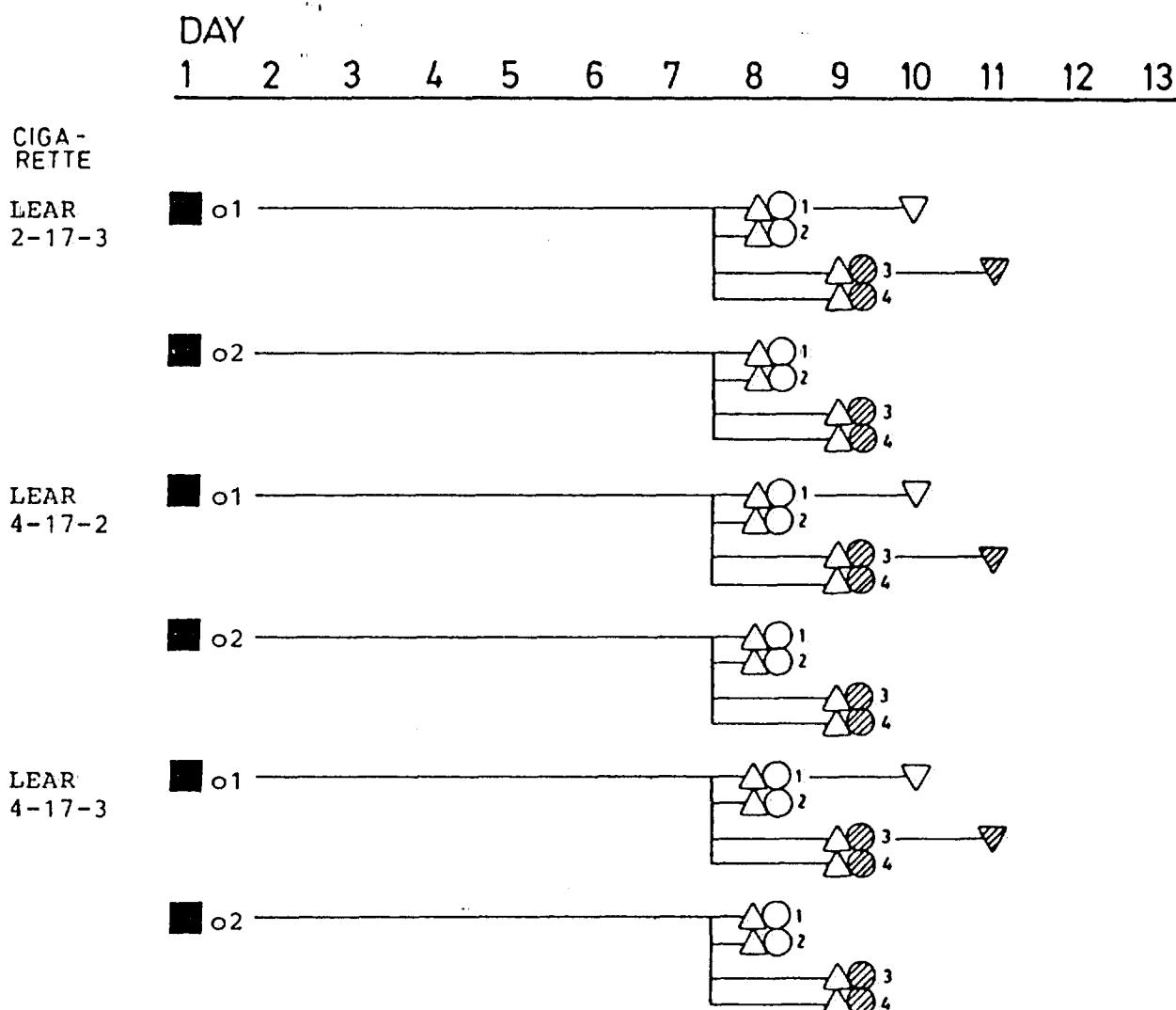


FIGURE A (continued)

CHRONOLOGICAL TABLE, ASSAY 1

WSC-I preparation (■), dilution of WSC-I suspension (△), mutagenicity assay with TA 98 (○) and with TA 100 (⊕) and reversion assay with TA 98 (▽) and with TA 100 (▷) of cigarette smoke condensates. The batch numbers of WSC-I suspension and dilution of WSC-I suspensions are shown beside the corresponding symbols.

Day 1: 2.Nov.81

2026048896

DAY

1 2 3 4 5 6 7 8 9 10 11 12 13

CIGA-
RETTE

2R1

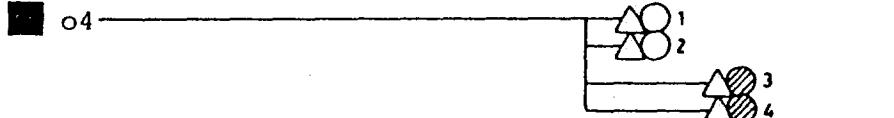
LEAR
o-17-2LEAR
o-17-3LEAR
2-17-2

FIGURE B

CHRONOLOGICAL TABLE, ASSAY 2

WSC-I preparation (■), dilution of WSC-I suspension (△), mutagenicity assay with TA 98 (○) and with TA 100 (⊖) and reversion assay with TA 98 (▽) and with TA 100 (▷) of cigarette smoke condensates. The batch numbers of WSC-I suspension and dilution of WSC-I suspensions are shown beside the corresponding symbols.

Day 1: 17.Nov.81

2026048897

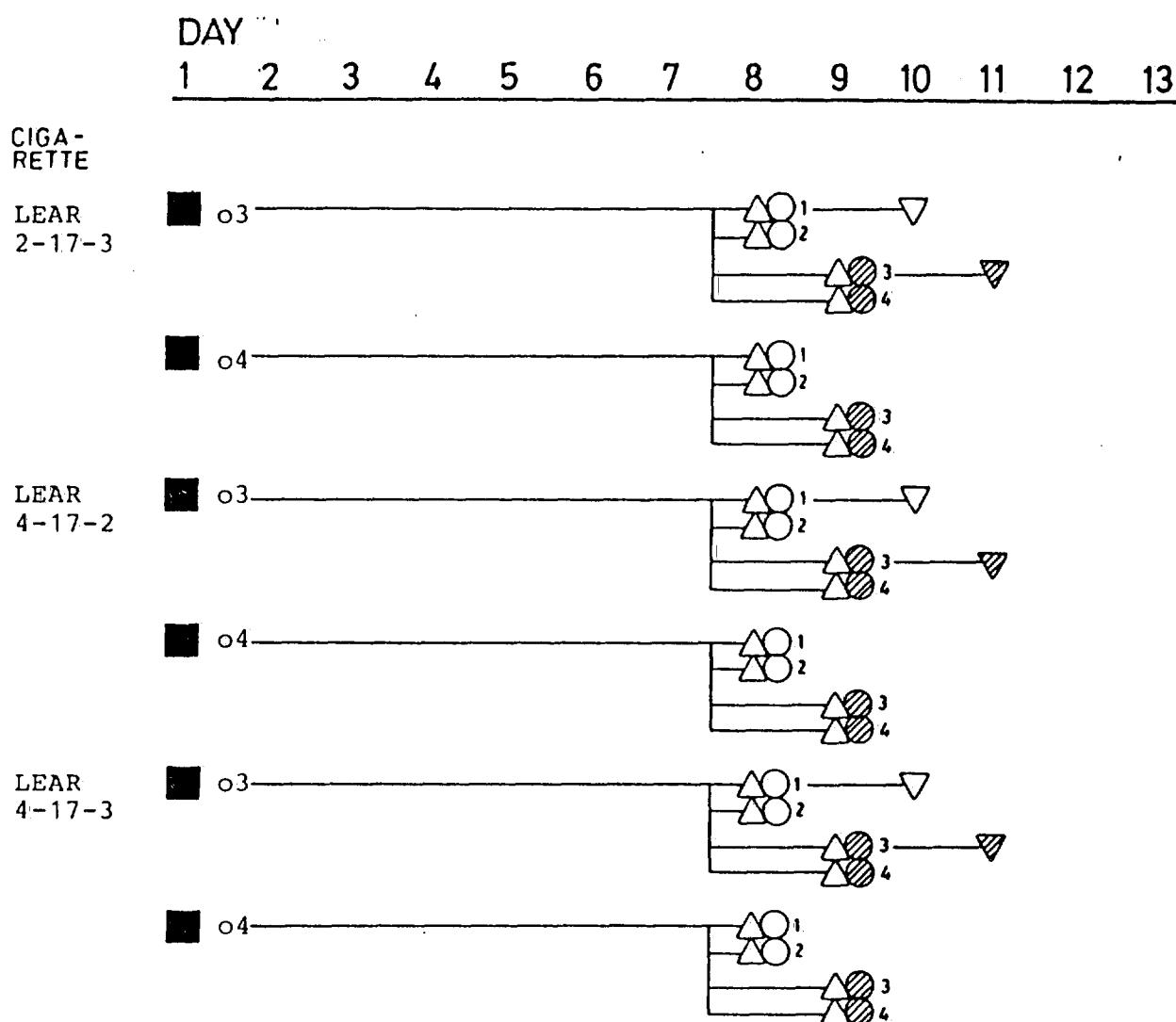


FIGURE B (continued)

CHRONOLOGICAL TABLE, ASSAY 2

WSC-I preparation (■), dilution of WSC-I suspension (△), mutagenicity assay with TA 98 (○) and with TA 100 (◎) and reversion assay with TA 98 (▽) and with TA 100 (▼) of cigarette smoke condensates. The batch numbers of WSC-I suspension and dilution of WSC-I suspensions are shown beside the corresponding symbols.

Day 1: 17.Nov.81

2026048898

Vacuum pump: rotary valve pump, Medvak MP 1,
Pfeiffer GmbH,
D-6330 Wetzlar

Rotameter: rotameter L 4/160,
Rota KG,
D-7867 Wehr/Baden

Soap-film flowmeter: Faust GmbH,
D-5000 Köln 90

Impaction trap

Type: glass "Impaction trap for cigarette
smoke condensate collection" according
to PM (see FIGURE C),
Faust GmbH,
D-5000 Köln 90

Capillary: length: 5 mm
bore: 0.4 mm

Mode of installation
of the impaction trap
insert: distance of 0.5 mm between capillary
tip and wall of flask calibrated with
0.5 mm thick teflon sheet spacer

Connection of impaction
trap to smoking machine:
so that the impaction trap lies
horizontally

Procedure

Puffs/cigarette: see TABLE 1

Puff frequency/cigarette: 1 puff/min

Puff duration: approx. 2 s minus time for change
of position

Puff volume: 35 ml
parameter checked and regulated
during condensation with rota-
meter or soap-film flowmeter

Scientific version: 14.Jan.81
Text version: 10.Aug.82

2026048899

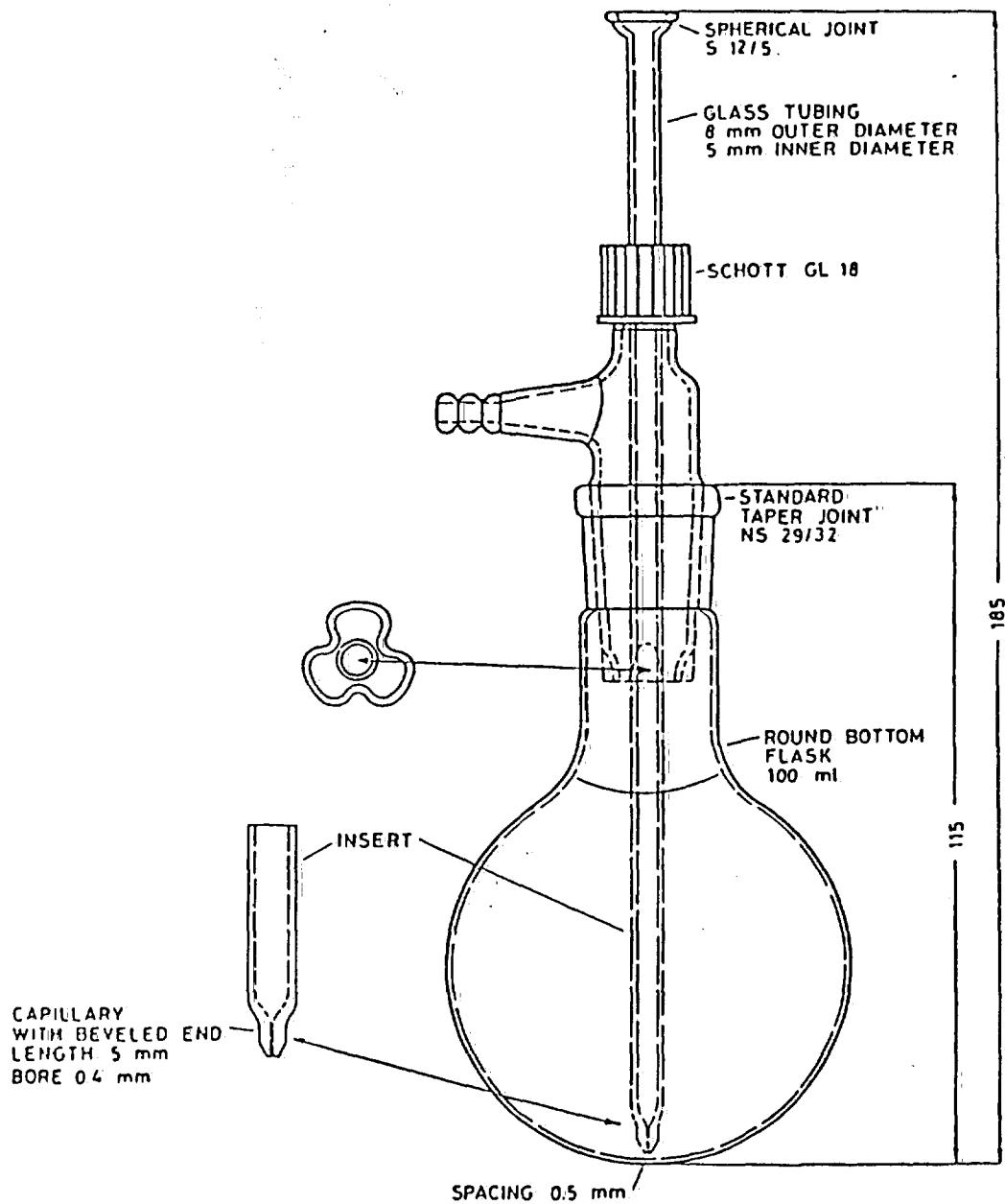


FIGURE C

IMPACTION TRAP

2026048900

4.2.2 Preparation and storage of WSC-I suspension

Principle: suspension of WSC-I in DMSO by sonication

Time: immediately after WSC-I preparation

Sample material and quantity: total WSC-I prepared from 30 cigarettes with 1 smoking machine

Equipment: sonication water bath, Sonorex RK 100, Bandelin KG, D-1000 Berlin

Chemicals and reagents: DMSO, for spectroscopy, no. 2950, E. Merck, D-6100 Darmstadt 1

Procedure: WSC-I washed out of impaction trap 6 times with approx. 3 ml portions of DMSO repeatedly after sonication (water bath) for approx. 3 min, washings transferred to a volumetric flask and filled up to 25 ml with DMSO

amount of WSC-I calculated from weight of impaction trap before and immediately after condensate preparation

amount of dry condensate (a) calculated from WSC-I and water concentration of suspension determination: see 4.2.3

determination of nicotine concentration: see 4.2.4

(a) The terms "moist condensate" and "dry condensate" are in accordance with DIN 10240 ("Maschinelles Abrauchen von Zigaretten, Bestimmung des feuchten und des trockenen Rauchkondensates").

2026048901

storage of WSC-I suspension:
in the dark, in sterile brown glass
bottles, 4 degrees centigrade

labeling of the bottles:
study no.,
batch no. (a),
dry condensate concentration (g/l),
date of condensate preparation

Scientific version: 15.Jan.81
Text version: 1.Oct.81

4.2.3 Determination of water concentration

Principle: titration according to Karl Fischer

Time: within 48 h after preparation of
WSC-I suspension

Sample material and quantity: WSC-I suspension (DMSO suspension),
1 ml, 2 determinations/suspension

Results expressed in: g/l

Equipment: Karl Fischer-Titrator E452,
Deutsche Metrohm GmbH,
D-7024 Filderstadt

Chemicals and reagents: Karl Fischer solution, no. 9248,
methanol, no. 6012,
DMSO, no. 2950,
E. Merck,
D-6100 Darmstadt 1

(a) Batch number consists of cigarette short code, type of condensate, consecutive number, date of condensate preparation, for example: 7A (I)/10/081280 (e. g.: code: 7A, condensate: I, batch no.: 10, date: 8.Dec.80).

2026048902

Procedure

Titration:

1 ml DMSO mixed with 4 ml methanol in the reaction vessel of the titrator and titrated with Karl Fischer solution to determine the water content of DMSO. Afterwards 1 ml cigarette smoke condensate suspended in DMSO titrated in the same way

Computation:

for computation the titer of the Karl Fischer solution determined by titration of a mixture of 1 ml DMSO and 4 ml methanol with a known amount of water, e. g. 10 mg

Detection limit:

0.5 g H₂O/l

Reproducibility

(rel. standard deviation): 2.8 % (10 g H₂O/suspension, N = 8)

Scientific version:

2.Jun.80

Text version:

23.Aug.82

4.2.4 Determination of nicotine concentration

Principle:

gas chromatography after extraction with dichloromethane

computer integration of peak areas

Time:

within 48 h after preparation of WSC-I suspension

Sample material and quantity:

WSC-I suspension (DMSO suspension), 1 ml, 2 determinations/suspension

Results expressed in:

g/l

2026048903

Equipment:

gas chromatograph: HP 5710 A,
detector: FID,
automatic sampler: HP 7671 A,
laboratory data system: HP 3351 A,
Hewlett-Packard GmbH,
D-6000 Frankfurt/Main

recorder: Servogor 210,
Metrawatt GmbH,
D-8500 Nürnberg

Chemicals and reagents:

nicotine, no. 77635,
Serva Feinbiochemica,
D-6900 Heidelberg

quinoline, no. 802407,
dichloromethane, no. 822271,
DMSO, no. 2950,
sodium hydroxide, no. 5594,
sulfuric acid (0.2 mol/l),
no. 9074,
E. Merck,
D-6100 Darmstadt 1

nitrogen,
hydrogen,
air (synthetic),
Linde AG,
D-5000 Köln 50

Procedure

Extraction:

addition of 1 ml of the internal
standard solution (0.5 mg quinoline/
ml 0.1 mol/l sulfuric acid), 1 ml
sodium hydroxide (200 g/l) and 10 ml
dichloromethane to 1 ml WSC-I
suspension, after agitation
(5 min) and centrifugation
(approx. 7.8×10^3 m/s² (= 800 x g),
5 min, approx. 10 degrees centi-
grade), injection of 1 μ l of the
lower organic phase into the gas
chromatograph

2026048904

Gas chromatography

Column: 2 m x 1/8 inch outer diameter,
stainless steel

Column packing: 10 o/o Apiezon L and 10 o/o KOH on
Chromosorb W-AW DMCS, 80 to 100 mesh

Carrier gas and
flow rate: nitrogen, 30 ml/min

Oven temperature
(degrees centigrade): 175

Injection port
temperature
(degrees centigrade): 200

Detector temperature
(degrees centigrade): 200

Computation: for computation 1 ml of a standard
solution (1 mg nicotine and 0.5 mg
quinoline/ml 0.1 mol/l sulfuric
acid) diluted with 1 ml DMSO and
extracted as described above

Detection limit: 0.02 g/l

Recovery: 98.5 o/o

Reproducibility
(rel. standard deviation): 0.5 o/o (1 g nicotine/l, N = 10)

Scientific version: 2.Jun.80
Text version: 29.Sep.81

2026048905

4.2.5 Dilution of WSC-I suspension

Principle: dilution of WSC-I suspension to defined dry condensate concentration

Time: 7 d after WSC-I preparation

Sample material and quantity: WSC-I suspension, approx. 3 ml

Equipment: Sonifier B-15, titanium microtip, Branson, Danbury, USA

Chemicals and reagents: DMSO for spectroscopy, no. 2950, E. Merck, D-6100 Darmstadt 1

Procedure: prior to dilution: sonication of WSC-I suspension for 20 s with 10 s interval, 60 % power (sonifier B-15);
2 dilutions per WSC-I suspension

Scientific version: 15.Aug.80
Text version: 1.Oct.81

4.2.6 Determination of bacterial contamination of test substance

Principle: determination of bacterial contamination of test substance assayed for mutagenicity in the plate incorporation assay
detection limited to aerobic bacteria growing on minimal-glucose agar plates

2026048906

Time: during plate incorporation mutagenicity assay

Sample material and quantity: test substance (highest dose/plate)

Results expressed in: colony forming units/plate

Equipment: incubator: model 3916,
Forma Scientific,
Marietta, Ohio, USA

petri dishes: no. 1029,
100 mm x 15 mm, polystyrole,
sterilized,
Falcon,
Oxhard, CA. 93030, USA

colony counter (manual): Colony
Star 2,
Funke-Gerber,
D-1000 Berlin

Chemicals and reagents: top agar and minimal-glucose agar,
composition: see 5.6 Plate Incorporation Mutagenicity Assay

Procedure: top agar and test substance mixed by
rotation and poured on minimal-glucose agar
2 plates/sample

incubation of 2 inoculated plates
at 37 degrees centigrade, manual
counting of colonies after 2 d of
incubation

Scientific version: 26.Jul.79
Text version: 29.Sep.81

2026048907

4.3 Preparation of Aroclor 1254-Induced Rat Liver Homogenate4.3.1 Animals

Species: albino rat

Strain: Sprague Dawley (caw-ico-wiga)

Type of breeding: outbred

Sex: male

Microbiological
conditions of breeding: SPFBreeder: WIGA Versuchstierzucht,
Willi Gassner GmbH und Co. KG,
D-8741 Sulzfeld

Transport containers: special filter cartons

INBIFO animal supply number: 120

Number of rats: required: 20
applied: 19

Date of shipment: 4.Aug.81

Age of rats (days): arrival: 45 + 2,
1st application: 49 + 2Body weight (grams): arrival: 186.8 + 1.5
administration: 206.6 + 1.7
section: 220.0 + 2.8

Acclimatization period (days): 4

Text version: 24.Aug.81

2026048308

4.3.2 Animal housing

Animal room: INBIFO main laboratory building,
barrier area SPF, room R513

Construction and interior: windowless

floors, walls and ceilings as well
as fixtures coated with epoxy resins

Microbiological conditions: SPF-barriered animal care

Conditioning and
ventilation: 100 % fresh air, delivered from a
50 m high air inlet stack, approx.
15 changes/h

filter: HEPA filter

Room temperature
(degrees centigrade): 22 ± 1

Relative humidity (%): 55 ± 10

Light: time cycle:
LD 12:12, L 7.00 to 19.00

source:
dampened "daylight" fluorescent lamps,
Universal Weiss,
Osram GmbH,
D-8000 München 1

intensity in cages:
approx. 20 to 100 Lux (a)

Cages: polycarbonate cages (Makrolon), type 3

base area: 43 cm x 17 cm
height: 15 cm

(a) higher intensity during handling of animals and cleaning
of rooms

2026048909

Cage lids: stainless steel lids with overhead hoppers

length: 44 cm

width: 23 cm

Bedding material: autoclaved granulated wood

sterilization:

15 min at 134 degrees centigrade

pressure: 2.03x10E5 Pa

replacement of bedding material:
2 times/week during application

Animals per cage: 2

Scientific version: 15.Jan.81

Text version: 29.Sep.81

2026048910

4.3.3 Food and drinking water

Food:

autoclaved fortified pellet food,
16 mm long, cylindrical pellets,
"HERILAN MRH-HALTUNG für Mäuse,
Ratten und Hamster" (a) (see PAGE
4-19),
H. Eggersmann KG,
D-3260 Rinteln/Weser

sterilization:

5 min at 120 degrees centigrade

pressure: 1.01x10E5 Pa

drying: 15 min

Food supply:

ad libitum from stainless steel
hoppers in cage lid, food removed
12 h before sacrifice

Water:

autoclaved tap water (a)

Water supply:

ad libitum from 250-ml DIN glass
bottles, with stainless steel sipper
tubes, water changed 2 times/week

Scientific version:

15.Jan.81

Text version:

9.Dec.81

(a) random samples of all autoclaved batches of food and of drinking water microbiologically investigated

2026048911

HERILAN MRH-HALTUNG

Alleinfutter für die Haltung ausgewachsener Mäuse,
Ratten und Hamster

Abpackungen: 50-, 25-kg-Papiersäcke oder Papiersäcke mit Polyaethylenenlage

Pressung: 16 mm, 10 mm, rund oder eckig

Energiegehalt: 10 500 joule umsetzbare Energie / kg Fertigfutter

Gehalt an Rohnährstoffen:
(% der lufttrockenen Substanz):

Rohprotein 16

Rohfett 4

Rohfaser 6

Gehalt an Mineralien und Spurenelementen:
(% der lufttrockenen Substanz):

Ca 0,80

P 0,70

Na 0,25

K 1,00

Mg 0,40

Fe min. 0,15

Mn min. 0,08

Cu min. 0,02

Zn min. 0,05

Vitaminzusatz pro kg Fertigfutter

Normalfutter Fortified-Futter

| | | |
|-------------------------|-------------|-------------|
| Vitamin A | 10 000 I.E. | 20 000 I.E. |
| Vitamin D ₃ | 400 I.E. | 600 I.E. |
| Vitamin E | 150 mg | 200 mg |
| Vitamin K ₃ | 10 mg | 15 mg |
| Vitamin C | 100 mg | 150 mg |
| Vitamin B ₁ | 20 mg | 30 mg |
| Vitamin B ₂ | 30 mg | 60 mg |
| Vitamin B ₆ | 20 mg | 30 mg |
| Vitamin B ₁₂ | 30 mcg | 40 mcg |
| Ca-Pantothenat | 40 mg | 60 mg |
| Nikotinsäureamid | 40 mg | 60 mg |
| Cholinchlorid | 1 000 mg | 1 200 mg |
| Folsäure | 5 mg | 6 mg |
| Inositol | 5 mg | 10 mg |
| Biotin | 80 mcg | 100 mcg |

Durchschnittlicher Gehalt an Aminosäuren:

(Angaben in g AS / kg Futter)

| | |
|----------------|------|
| Alanin | 3,2 |
| Arginin | 22,3 |
| Asparaginsäure | 4,5 |
| Cystin | 15,4 |
| Glutaminsäure | 11,3 |
| Glycin | 18,1 |
| Histidin | 8,2 |
| Iso-Leucin | 12,6 |
| Leucin | 24,0 |
| Lysin | 8,6 |
| Methionin | 3,1 |
| Phenylalanin | 3,3 |
| Prolin | 4,3 |
| Serin | 2,9 |
| Threonin | 14,0 |
| Tryptophan | 5,1 |
| Tyrosin | 16,3 |
| Valin | 17,6 |

SPECIFICATIONS BY SUPPLIER

2026048912

4.3.4 Administration of Aroclor 1254

Principle: intraperitoneal injection

Time: 5 d prior to sacrifice

Sample material and quantity: rats, 19

Equipment: sonifier: cell disruptor B-15,
titanium microtip,
Branson,
Danbury, USAsyringes: 2 ml, sterile, no. 9410002,
cannula: sterile, no. 9410118,
Hirtz and Co.,
D-5000 Köln 51Chemicals and reagents: Aroclor 1254,
Dr. S.u.I. Ehrenstorfer,
D-8900 Augsburgcorn oil: Mazola,
Maizena GmbH,
D-7100 Heilbrunn

Procedure

Preparation: Aroclor emulsified in corn oil
by sonication (5 x 10 s, 0 degrees centigrade) to a concentration of 200 mg/ml

Dose: 500 mg/kg BW

Scientific version: 25.Jan.80
Text version: 29.Sep.81

2026048913

4.3.5 Preparation of tissue homogenate supernatant (S9 fraction)

Principle: mechanical grinding and centrifugation of tissue

Time of sacrifice: 5 d after administration of Aroclor

Sample material and quantity: livers, 19 pooled

Final product: Aroclor 1254-induced rat liver homogenate, batch no. 81.A

Equipment:

- Potter-Elvehjem glass homogenizer with motor-driven teflon pestle, Braun Melsungen GmbH, D-3508 Melsungen
- Multifix, model M 80, Alfred Schwinher, D-7070 Schwäbisch-Gmünd
- centrifuge: Sorvall RC-5B, rotor: SS-34, Du Pont Instruments, D-6350 Bad Nauheim
- freezer: Forma Scientific, no. 8218, Forma Scientific, Marietta, Ohio, USA
- tubes: polycarbonate, no. 3137, polypropylene, no. 3138, Du Pont Instruments, D-6350 Bad Nauheim
- polypropylene, no. 3810, Netheler and Hinz, D-2000 Hamburg 63
- glassware and equipment in contact with homogenate precooled and sterile

Chemicals and reagents:

- potassium chloride, no. 4936, E. Merck, D-6100 Darmstadt 1

2026048914

Procedure:

according to Ames et al., Mutation Research 31: 347-364 (1975)

killing:

decapitation, followed by 30 s exsanguination

removal of organs:

sterile dissection after soaking the fur with ethanol (700 ml/l), short submersion of removed organs in 150 mmol/l potassium chloride, pH 7.0, determination of WW

homogenization:

after addition of 3 volumes of 150 mmol/l potassium chloride, pH 7.0, to the original organ weight (1 g equiv. to 1 ml) mechanical grinding in Potter-Elvehjem apparatus with motor-driven teflon pestle, approx. 200 rpm

centrifugation:

8.83×10^4 m/s² (= 9000 x g), 10 min, 4 degrees centigrade

storage of supernatant

(S9 fraction):

minus 80 degrees centigrade, 1-ml and 5-ml aliquots

Scientific version:

7.Dec.80

Text version:

30.Dec.81

4.4 Analyses of Rat Liver Homogenates

4.4.1 Determination of protein concentration (Biuret method)

Principle:

photometric determination of a dye complex formed between peptide bonds and the Biuret reagent

2026046915

Time: 26.Nov.81

Sample material and quantity: S9 fraction, 10 µl

Results expressed in: g/l

Equipment: ABA-100 Bichromatic Analyzer,
Abbott Laboratories, Diagnostics
Division,
So. Pasadena, California 91030, USAChemicals and reagents: test set Total protein (Biuret
method), no. 124281,
standard protein: Precinorm S,
no. 125121,
Boehringer Mannheim GmbH,
D-6800 Mannheim 31Procedure: according to Weichselbaum, T.E.,
Amer. J. Clin. Path. 16: 40 (1946),
adapted by INBIFO to the bichro-
matic analyzer

photometric determination:

wavelength 1: 550 nm
wavelength 2: 650 nmeach sample determined in dupli-
cates

standard curve: see FIGURE D

Scientific version: 28.Mar.79
Text version: 27.Nov.814.4.2 Determination of aryl hydrocarbon monooxygenase (EC 1.14.14.2)
activityPrinciple: fluorometric determination of 3- and
9-OH-B(a)P formed during the incuba-
tion of B(a)P with tissue extracts
and separated after incubation by
HPLC

2026048916

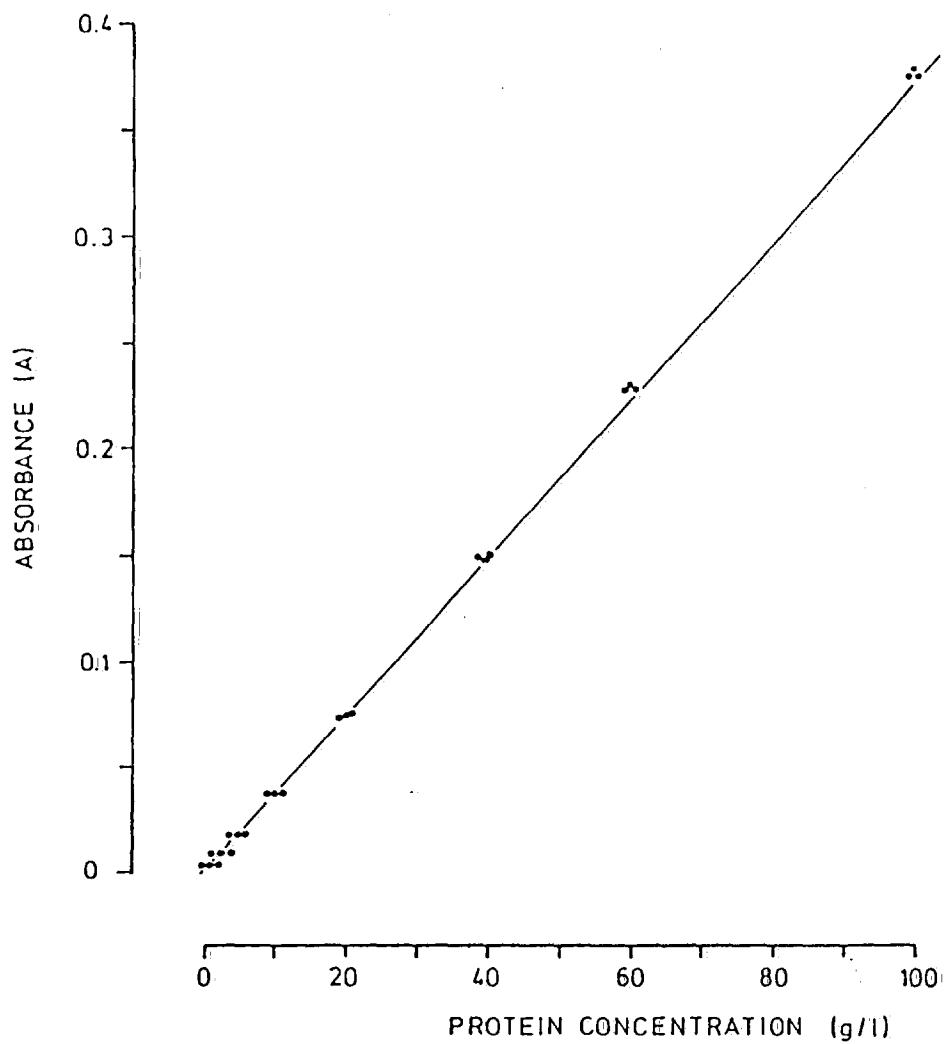


FIGURE D

PROTEIN STANDARD CURVE, BIURET METHOD

Scientific version: 28.Mar.79

2026048917

Time: 26.Nov.81

Sample material and quantity: liver: S9-fraction,
approx. 6 ug of proteinResults expressed in: U/mg protein
(1 U = (1 nmol 3- plus 9-OH-B(a)P)/h)Equipment: rotary mixer:
Cenco Instrumenten,
Breda, The Netherlandshigh pressure liquid chromatograph:
model 1084 B,
Hewlett Packard,
D-7500 Karlsruhecolumn: RP-18, 250 mm x 4.6 mm,
Knauer,
D-6370 Oberurselincubation vessels: no. 611-52,
Sovirel,
Le Vallois Perret, Franceinjection vial: no. 80184, 11.5 mm
x 325 mm,
Müller und Müller Glaswarenfabrik,
D-3450 Holzmindenspectrofluorometer: model 650-10S,
Perkin Elmer GmbH,
D-7770 Überlingenshaking water bath: no. 3047,
Köttermann,
D-3165 Hänigsencentrifuge: Sorvall RC-5B, rotor:
SS-34,
polyallomer tubes, no. 3128,
Du Pont Instruments,
D-6350 Bad NauheimChemicals and reagents: magnesium chloride, no. 5833,
acetonitrile, no. 30,
monosodium phosphate buffer, pH 4.8,
no. 6346,
acetone, no. 14,
methanol, no. 6007,
E. Merck,
D-6100 Darmstadt 1

2026046918

glucose-6-phosphate dehydrogenase
(G6PDH, EC 1.1.1.49), no. 197726,
glucose-6-phosphate (G6P), no. 127027,
NADP, no. 128031,
NAD, no. 127981,
Boehringer Mannheim GmbH,
D-6800 Mannheim 31

benzo(a)pyrene (B(a)P), no. 3176,
chinine sulfate (standard solution:
1.1 ug/ml 0.05 mol/l sulfuric acid),
no. 2-8838,
Carl Roth GmbH,
D-7500 Karlsruhe 21

bovine serum albumin (BSA), no.
A 4378,
tris(hydroxymethyl)aminomethane
(Tris), no. T 1503,
Sigma Chemie GmbH,
D-8021 Taufkirchen

9-hydroxybenzo(a)pyrene, no. 106,
3-hydroxybenzo(a)pyrene, no. 75,
IIT Research Institute,
Chicago Illinois, 60616, USA

Procedure

Enzyme incubation:

according to Van Cantfort, J.,
De Graeve, J. and Gielen, J.E.,
Biochem. Biophys. Res. Commun. 79:
505-512 (1977)

incubation time: 10 min

final concentrations of components
in assay mixture:

| | |
|---------------------|--------------|
| S9 fraction protein | 12.00 mg/l |
| B(a)P | 0.08 mmol/l |
| NAD | 0.43 mmol/l |
| NADP | 0.37 mmol/l |
| G6P | 2.50 mmol/l |
| BSA | 0.80 g/l |
| Tris-HCl, pH 7.6 | 50.00 mmol/l |
| magnesium chloride | 5.00 mmol/l |
| G6PDH | 1 U/ml |

total assay volume: 0.50 ml

triplicate determination per sample

2026048919

HPLC separation

Injection volume: 20 μ l

Column: RP-18, particle diameter: 10 μ m, column length: 250 mm, inner diameter: 4.6 mm

Oven and solvent temperature: 40 degrees centigrade

Solvent A: 500 ml/l acetonitrile
500 ml/l 10 mmol/l sodium dihydrogen phosphate buffer, pH 4.8

Solvent B: 1000 ml/l acetonitrile

Gradient: linear increase from 800 ml/l A and 200 ml/l B to 100 ml/l A and 900 ml/l B within 20 min
resulting gradient: 600 to 950 ml/l acetonitrile

Flow rate: 0.8 ml/min

Column pressure: 70 to 100 bar

Detection: fluorometric, excitation wavelength: 305 nm, emission wavelength: 430 nm, slit of excitation and emission monochromators: 15 nm

Calculations: standard curve: see FIGURE E

Scientific version: 4.Feb.81
Text version: 10.Aug.81

4.4.3 Determination of bacterial contamination of S9 fraction

Principle: determination of bacterial contamination by growth on nutrient agar plates
detection limited to aerobic cocci and rods

Time: immediately after preparation of

2026048920

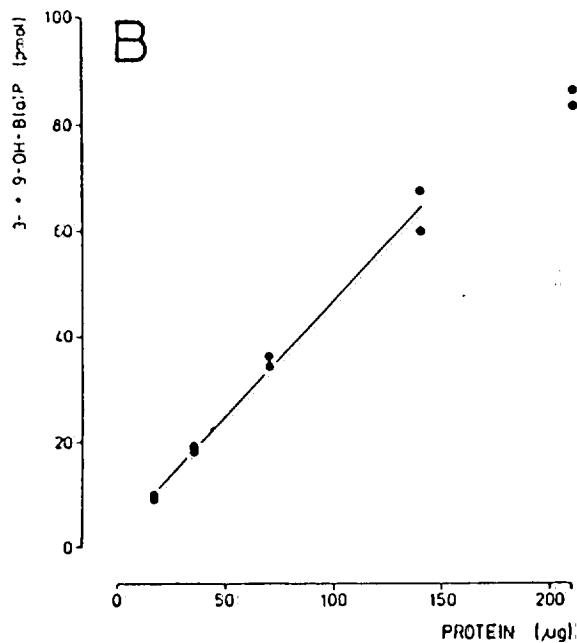
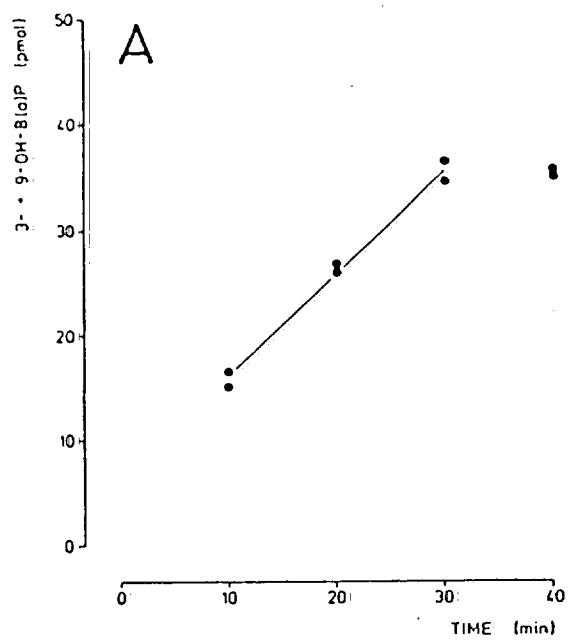


FIGURE E

DETERMINATION OF AHM ACTIVITY

Remarks: A: time response, 70 ug protein (a)

B: protein response (a), 30 min incubation time

date of determination: 19.Feb.81

scientific version: 4.Feb.81

(a) 9.8×10^5 m/s² microsomal pellet after dermal application of 100 μ l acetone/mouseSource: <https://www.industrydocuments.ucsf.edu/docs/gsmm0000>

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Sample material and quantity: liver S9 fraction, 0.1 ml/plate

Results expressed in: CFU/ml

Equipment: incubator: model 3916,
Forma Scientific,
Marietta, Ohio, USA

petri dishes: no. 1029,
100 mm x 15 mm, polystyrole,
sterilized,
Falcon,
Oxhard, CA. 93030, USA

colony counter: Colony Star 2,
Funke-Gerber,
D-1000 Berlin

Chemicals and reagents: nutrient agar, standard 1, no. 7881,
E. Merck,
D-6100 Darmstadt 1

Procedure: incubation of 2 inoculated plates at
37 degrees centigrade

manual counting of colonies after
3 d of incubation

Scientific version: 26.Jul.79

Text version: 21.Sep.81

4.4.4 Determination of promutagen activation

Principle: plate incorporation mutagenicity
assay with standard mutagens in
the presence of various doses
of S9 protein

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Sample material and quantity: S9 fraction 81.A, approx. 0.4 to 5 mg protein/plate

Results expressed in: revertants/plate

Equipment: see 4.6 Plate Incorporation Mutagenicity Assay

Chemicals and reagents: standard mutagens:
benzo(a)pyrene, no. 3176,
Carl Roth GmbH,
D-7500 Karlsruhe

2-aminoanthracene (2-AA), no. A3,880-0,
Aldrich Europe,
D-4054 Nettetal 2

2-aminofluorene (2-AF), no. 820573,
Merck-Schuchardt,
D-8011 Hohenbrunn

WSC-I of 2R1, batch no. 49,
2.5 mg dry condensate/ml DMSO

DMSO, for spectroscopy, no. 2950,
E. Merck,
D-6100 Darmstadt 1

concentrations of S9 mix components
prior to filtration and dilution:

| | |
|-------------------------------------|--------------|
| MgCl ₂ 6-hydrate | 16.0 mmol/l |
| KCl | 66.6 mmol/l |
| sodium phosphate buffer (pH 7.4) | 100.0 mmol/l |
| glucose-6-phosphate-Na ₂ | 5.0 mmol/l |
| NADP-Na ₂ 3-hydrate | 4.0 mmol/l |
| S9 protein | 10.0 g/l |

top agar and minimal-glucose agar: see
4.6 Plate Incorporation Mutagenicity
Assay

Salmonella typhimurium, tester strains
see 4.5.1 Nutrient broth suspension
culture

2026048923

Procedure:

filtration of S9 mix prior to dilution, dilution with sodium phosphate buffer, 0.1 mol/l, pH 7.4

plating of S9 mixes in plate incorporation mutagenicity assay with standard mutagens, 4 plates per S9 mix dose and standard mutagens

doses of standard mutagens:

WSC-I 0.1 mg/plate
2-AA 2 ug/plate
2-AF 2 ug/plate
B(a)P 5 ug/plate

incubation and counting of revertants:
see 4.6 Plate Incorporation Mutagenicity Assay

Scientific version:

26.Jun.80

Text version:

10.Aug.82

4.5 Bacterial Tester Strains

4.5.1 Nutrient_broth_suspension_culture

Principle:

overnight cultivation of tester strain bacteria in complete nutrient broth

Time:

12 h prior to harvest

Sample material and quantity: *Salmonella typhimurium*, tester strains TA 98 and TA 100 (a), frozen stock cultures

Equipment:

incubator shaker: model G 24, New Brunswick Scientific, Edison, New Jersey, USA

(a) kindly provided by Prof. Dr. Bruce Ames, University of California, Berkeley CA., USA

2026048924

culture flask: Erlenmeyer flask,
100 ml, with long neck, used with
stainless steel caps,
Schott,
D-6500 Mainz

cryo tubes with screw caps, 2.0 ml,
no. 363401,
Nunc GmbH,
D-6200 Wiesbaden

Chemicals and reagents: Difco-Bacto nutrient broth,
no. 0003-01,
Difco Laboratories,
Detroit, Michigan 48201, USA

DMSO, for spectroscopy, no. 2950,
E. Merck,
D-6100 Darmstadt 1

Procedure

Preparation and growth:

inoculation of 30 ml nutrient broth
in culture flask with 10 µl of the
thawed and 10-fold diluted stock cul-
ture, cultures grown in a shaking
incubator at 37 degrees centigrade
with 200 rpm for 12 h

Storage of frozen stock culture:

aliquots (0.1 ml) of tester strain
suspensions in nutrient broth with
87.5 µl DMSO/l at minus 196 degrees
centigrade in liquid nitrogen

Scientific version:
Text version:

12.Jan.81
29.Sep.81

2026048925

4.5.2 Determination of the density of bacteria suspension culture

Principle: photometric determination of the amount of light scattered by the suspension of bacteria

Time: at the beginning and at the end of the plate incorporation assay

Sample material and quantity: tester strain suspension culture, 1.0 ml

Results expressed in: absorbance unit (A)

Equipment: photometer: Spectronic 20, colorimeter test tubes, Bausch und Lomb, D-8043 Unterföhring

Chemicals and reagents: NaCl, no. 6400, E. Merck, D-6100 Darmstadt 1

Procedure: dilution:
1.0 ml suspension culture + 4.0 ml NaCl (9 g/l)
photometric determination:
wavelength: 565 nm
blank: NaCl (9 g NaCl/l)
absorbance calculation for the undiluted culture

Scientific version: 9.May 80
Text version: 21.Sep.81

2026048926

4.5.3 Determination of the number of viable bacteria

Principle: spreading of bacteria with top agar plating technique and counting of colony forming units

Time: immediately prior to start and at the end of each plate incorporation assay

Sample material and quantity: tester strain suspension culture, approx. 0.1 ml

Results expressed in: CFU/ml

Equipment: colony counter: Artek counter, model 880, Artek System, via Fisher Scientific, D-8000 München

incubator: model 3916, Forma Scientific, Marietta, Ohio, USA

petri dishes: no. 1029, 100 mm x 15 mm, polystyrole, sterilized, Falcon, Oxhard, CA. 93030, USA

Chemicals and reagents: minimal-glucose agar and top agar, composition: see 4.6 Plate Incorporation Mutagenicity Assay

sodium chloride, no. 6400, E. Merck, D-6100 Darmstadt 1

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L-histidine hydrochloride 1-hydrate,
no. H 8125,
biotin, no. B 4501,
Sigma Chemie GmbH,
D-8021 Taufkirchen

histidine/biotin solution:
19.17 g histidine hydrochloride 1-hydrate and 24.4 mg biotin dissolved in H₂O, sterilized by filtration

Procedure:

aliquots of tester strain suspension culture diluted 10⁶-fold in 9 g/l NaCl. 0.1 ml of this dilution mixed with 2.0 ml top agar and 0.1 ml histidine/biotin solution and plated on minimal-glucose agar plates

incubation:

48 h at 37 degrees centigrade

counting:

manual and/or automatic counting of colony forming units

Scientific version:

13.Jan.81

Text version:

8.Jul.82

4.5.4 Analyses_of_tester_strain_properties

Principle:

tester strain checked for:

- (1) auxotrophy in the form of histidine requirement
- (2) absence of lipopolysaccharide barrier in the form of sensitivity to crystal violet
- (3) presence of R factor in the form of resistance to ampicillin
- (4) lack of excision repair system in the form of sensitivity to UV light

2026048928

Time: at the beginning and at the end of each study

Sample material and quantity: tester strain suspension culture, approx. 0.5 ml

Equipment: UV source: Astralux F, no. 15136, 890 W, Astralux-Werke, Vienna, Austria

filter paper disks: no. 2668, diameter: 6 mm, Schleicher und Schüll, D-3354 Dassel

petri dishes: no. 1029, polystyrole, Falcon, via Becton Dickinson GmbH, D-6900 Heidelberg 1

incubator: model 3916, Forma Scientific, via Labotect, D-3400 Göttingen

Chemicals and reagents: L-histidine hydrochloride 1-hydrate, no. H 8125, biotin, no. B 4051, Sigma Chemie GmbH, D-8021 Taufkirchen

crystal violet, no. 1407, nutrient agar, standard 1, no. 7881, E. Merck, D-6100 Darmstadt 1

ampicillin sensitivity disk, 10 µg, Oxoid Deutschland GmbH, D-4230 Wesel

minimal-glucose agar and top agar, composition: see 4.6 Plate Incorporation Mutagenicity Assay

2026048929

Procedure of test for

Histidine requirement:

tester strain bacteria streaked on minimal-glucose agar plate without and with 0.1 ml of histidine and biotin solution (0.1 mol L-histidine hydrochloride 1-hydrate and 0.5 mmol biotin/l)

incubation at 37 degrees centigrade for 18 to 24 h

plates checked for growth

Crystal violet sensitivity:

10 µl crystal violet solution (1 g/l) applied to filter paper disk, placed onto complete nutrient agar plate and overlayed with 0.1 ml tester strain suspension culture

incubation at 37 degrees centigrade for 12 to 16 h

plate checked for inhibition zone

Ampicillin resistance:

ampicillin disk (10 µg) applied onto complete nutrient agar with tester strain bacteria plated

incubation at 37 degrees centigrade for 18 to 24 h

plate checked for growth around ampicillin disk

UV sensitivity:

tester strain bacteria to be tested streaked across nutrient agar plates and half of the streak irradiated for 30 s with a UV source at a distance of 10 cm

incubation at 37 degrees centigrade for 18 to 24 h

plate checked for growth inhibition

Scientific version:

20.May 81

Text version:

4.Aug.82

2026048930

4.6 Plate Incorporation Mutagenicity Assay

Principle: counting of revertants after growth in the presence of test substance

Time of top agar plating: see FIGURES A and B

Sample material and quantity: (1) 2-AA and 2-AF, 0.05 g/l,
2 ug/plate
(2) test substance: WSC-I of test cigarette types PRO-3 and PRO-4
(3) reference substance: standard reference cigarette WSC-I

Results expressed in: revertants/plate

Equipment: sterile filter: Millex SLHA,
no. 0250 S and HAWG, no. 04700,
0.45 um pore size,
Millipore S.A.,
F-6700 Molsheim, France

incubator: model 3916,
Forma Scientific,
Marietta, Ohio, USA

whirlmix: no. 34524-200,
Cenco Instrumenten,
Breda, The Netherlands

thermostat: aluminium bloc thermostat, no. 2092,
Gebr. Liebisch,
D-4800 Bielefeld

test tubes: no. 3033, 16 mm x 125 mm,
polystyrole, sterile,
Falcon,
Oxhard, CA. 93030, USA

colony counter (manual): Colony Star 2,
Funke-Gerber,
D-1000 Berlin

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colony counter (automatic): Artek counter, model 880,
Artek System,
Farmingdale, N.Y., USA

automatic pipettes:

- (1) Cornwall refilling syringes, volume: 2 ml (for top agar), E. Schütt, D-3400 Göttingen
- (2) bottle-top dispenser "dispensette", volume: 2 ml (for S9 mix), R. Brand GmbH + Co., D-6980 Wertheim
- (3) bottle-top dispenser "dis-trivar", volume: 0.5 ml (for bacteria suspension), Gilson France, S.A., F-95400 Villiers le Bel
- (4) adjustable pipettes: P 20, P 200 and P 1000, volume: 0.02, 0.2 and 1 ml (for test substances), Gilson France, S.A., F-95400 Villiers le Bel

Chemicals and reagents:

DMSO, for spectroscopy, no. 2950,
E. Merck,
D-6100 Darmstadt 1

2-aminoanthracene (2-AA), no. A 3,880-0,
Aldrich Europe,
D-4054 Nettetal 2

2-aminofluorene (2-AF), no. 820573,
Merck-Schuchardt,
D-8011 Hohenbrunn

composition of minimal-glucose agar,
top agar and S9 mix: see TABLES E,
F and G

Salmonella typhimurium tester strains
see 4.5.1 Nutrient broth suspension
culture

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| COMPOUND | ART. NO. | SOURCE | CONCENTRATION (g/l) |
|--|----------|-----------|------------------------|
| agar | 0140-01 | Difco (a) | 15.0 |
| glucose 1-hydrate | 8342 | Merck (b) | 20.0 |
| MgSO ₄ 7-hydrate | 5886 | Merck (b) | 0.2 |
| K ₂ HPO ₄ 3-hydrate | 5099 | Merck (b) | 13.1 |
| citric acid 1-hydrate | 244 | Merck (b) | 2.0 |
| NaNH ₄ HPO ₄ 4-hydrate | 6682 | Merck (b) | 3.5 |

TABLE E

COMPOSITION OF MINIMAL-GLUCOSE AGAR

preparation:

salts and glucose as 10-fold concentrated solutions separately

sterilization:

1 bar, 121 degrees centigrade, 15 min, except glucose, which was added to the molten medium as a sterile-filtered solution

filling into petri dishes (c):

automatically, approx. 30 ml molten agar/plate. Drying: excess water on the solid agar plates removed by exposure of the covered plates at 37 degrees centigrade for 3 d

storage of minimal-glucose agar plates:
after drying at RT

(a) Difco Laboratories, Detroit, Michigan, USA

(b) E. Merck, D-6100 Darmstadt 1

(c) automatic petri dish filler, Laboramat II,
Labora, D-6395 Weilrod-Winden

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| COMPOUND | ART. NO. | SOURCE | CONCENTRATION (g/l) |
|---|----------|-----------|------------------------|
| agar | 0140-01 | Difco (a) | 0.55 |
| sodium chloride | 6400 | Merck (b) | 0.45 |
| L-histidine hydrochloride 1-hydrate | H 8125 | Sigma (c) | 0.0087 |
| biotin | B 4501 | Sigma (c) | 0.011 |

TABLE F

COMPOSITION OF TOP AGAR

preparation:

histidine and biotin prepared as a 10-fold concentrated solution

sterilization:

- (1) histidine/biotin solution: sterile filtration
- (2) agar/sodium chloride: 1 bar, 121 degrees centigrade, 15 min

storage:

- (1) histidine/biotin at 4 degrees centigrade
- (2) agar/sodium chloride at RT

prior to use:

agar/sodium chloride remolten by boiling in a water bath for approx. 20 min, addition of histidine/biotin after cooling down to approx. 45 degrees centigrade

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(a) Difco Laboratories, Detroit, Michigan, USA

(b) E. Merck, D-6100 Darmstadt 1

(c) Sigma Chemie GmbH, D-8021 Taufkirchen

| COMPOUND | ART. NO. | SOURCE | CONCENTRATION |
|-------------------------------------|---------------|----------------|---------------|
| MgCl ₂ 6-hydrate | 5833 | Merck (a) | 6.4 mmol/l |
| KCl | 4936 | Merck (a) | 26.4 mmol/l |
| sodium phosphate buffer, pH 7.4 | 6346 and 6580 | Merck (a) | 100 mmol/l |
| glucose-6-phosphate-Na ₂ | 127o27 | Boehringer (b) | 2.3 mmol/l |
| NADP-Na ₂ 3-hydrate | 128o58 | Boehringer (b) | 1.6 mmol/l |
| S9 protein | - | - | 4.0 g/l (c) |

TABLE G

COMPOSITION OF S9 MIX

sterilization:

magnesium chloride/potassium chloride and sodium phosphate buffer are sterilized separately: 1.02 x 10⁵ Pa, 121 degrees centigrade, 15 min

glucose-6-phosphate and NADP: filter-sterilized (pore size: 0.45 um)

storage:

magnesium chloride/potassium chloride and sodium phosphate buffer: at 4 degrees centigrade

glucose-6-phosphate and NADP at minus 80 degrees centigrade

prior to use:

sterile filtration of the complete S9 mix

(a) E. Merck, D-6100 Darmstadt 1

(b) Boehringer Mannheim GmbH, D-6800 Mannheim 31

(c) concentration of S9 protein prior to filtration

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Procedure**Plating mixture preparation:**

components added in the following order:

- (1) 2.0 ml top agar, 45 degrees centigrade
- (2) test substance, 2 plates/dose,
- (3) 0.1 ml tester strain suspension culture, containing approx. 10^8 viable bacteria at 0 degrees centigrade
- (4) 0.5 ml S9 mix or S9 mix buffer at 0 degrees centigrade

Top agar plating:

components mixed by rotating the test tube gently on a whirlmix, then poured on minimal-glucose agar plates and spread evenly on the surface by wobbling. The mixing, pouring and spreading of the top agar occurred within 20 s. The plates allowed to harden for 3 to 6 min and then transferred to the dark incubator

Incubation:

48 h at 37 degrees centigrade in the dark

Counting of revertants:

if determination of the number of revertants not performed immediately at the end of the incubation period, plates stored at 4 degrees centigrade for not longer than 24 h

if necessary, plates brought to RT and the number of revertants counted manually and/or automatically

automatic counting:

each plate counted 3 times, rotation of the plate for 120 degrees between each count, highest count used for the calculation of revertants

Scientific version:
Text version:

3.Jul.80
30.Sep.81

2026048936

4.7 Spot Assay**Principle:**counting of revertants after growth
in the presence of diagnostic muta-
gens applied to filter paper disk**Time:**at the day of plate incorpora-
tion mutagenicity assay**Sample material and quantity:** (1) 9-aminoacridine, 0.5 g/l ethanol,
10 µg/plate
(2) MMS, 2 µl/plate
(3) MNNG, 0.1 g/l DMSO, 2 µg/plate**Results expressed in:**

revertants/plate

Equipment:incubator: model 3916,
Forma Scientific,
via Labotect,
D-3400 Göttingencolony counter (automatic): Artek
counter, model 880,
Artek System,
via Fisher Scientific,
D-8000 Münchenfilter paper disks: no. 2668, dia-
meter: 6 mm,
Becton Dickinson GmbH,
D-6900 Heidelberg**Chemicals and reagents:**dimethyl sulfoxide (DMSO),
for spectroscopy, no. 2950,
ethanol, no. 972,
E. Merck,
D-6100 Darmstadt 19-aminoacridine, no. 3,840-1,
N-Methyl-N'-nitro-N-nitrosoguanidine
(MNNG), no. 12,994-1,
Ega Chemie,
D-7924 Steinheim

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methyl methanesulfonate (MMS),
no. 12,992-5,
Aldrich Europe,
D-4054 Nettetal 2

Salmonella typhimurium tester strains:
see 4.5.1 Nutrient broth suspension
culture

top agar and minimal-glucose agar,
composition: see 4.6 Plate Incorpora-
tion Mutagenicity Assay

Procedure:

top agar plating of tester strains on
minimal-glucose agar

diagnostic mutagens applied to filter
paper disks placed on top of hardened
agar plates

incubation:
48 h at 37 degrees centigrade in the
dark

automatic counting of revertants:
see 4.6 Plate Incorporation Mutagenicity Assay

Scientific version:

3.Jul.79

Text version:

3.Jun.82

4.8 Reversion Assay

Principle:

analysis of growth of individual
colonies on minimal-glucose agar
without histidine

Time:

2 d after start of plate incor-
poration assay

2026048938

Sample material and quantity: 10 individual colonies from 1 mutagenicity assay plate with the highest dose of test substance

Results expressed in: number of colonies grown (histidine prototroph/10 colonies inoculated)

Equipment: incubator: model 3916,
Forma Scientific,
Marietta, Ohio, USA

Chemicals and reagents: minimal-glucose agar, composition:
see 4.6 Plate Incorporation Mutagenicity Assay

Procedure: streaking of revertant colonies on minimal-glucose agar plate with a platinum loop, incubation of plates at 37 degrees centigrade for 48 h and counting of colonies grown after incubation

Scientific version: 25.Mar.80
Text version: 30.Sep.81

4.9 Statistical Evaluation

Primary data (revertants/plate): calculation of MEAN and RSD from all plates for each cigarette, also calculated separately for assay 1 and assay 2, data not corrected for automatically counting

Specific mutagenicity: equivalent to regression coefficient "a" of a linear dose-response curve $y = ax + b$

2-AF: increase of revertants per ug 2-AF

WSC-I: increase of revertants per mg dry condensate

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Relative specific mutagenicity:

mutagenicity of WSC-I of cigarettes related to the mutagenicity of the standard reference cigarette 2R1 prepared and assayed in the same assay (internal standard set to 100 percent)

Relative difference:

absolute difference between 2 values (A and B) divided by the mean of them:

$$\frac{|A - B|}{(A + B)/2}$$

Statistical significance of the difference between the specific mutagenicities of 2 independent assays:

statistical significance reached at the level of significance equal to 0.017 with a relative difference of the specific mutagenicities by 25 percent (a)

Statistical significance of the difference between the specific mutagenicities of 2 individual cigarettes:

statistical significance reached at the level of significance equal to 0.05 with a relative difference of the specific mutagenicities by 16 percent (a)

Scientific version:
Text version:

21.Jul.81
25.Aug.82

(a) In a basic biometric study with tester strain TA 98 a single cigarette type was assayed according to the INBIFO WSC-I standard mutagenicity assay procedure (same procedure as in the present study: 2 independent assays, 4 doses and 64 plates/cigarette type). For the lack of basic biometric study with tester strain TA 100, the limit of the relative difference for 2 cigarettes or 2 assays is set to 0.16 or 0.25 respectively following the biometric study with TA 98.

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5 STORAGE OF MATERIALS AND RECORDS

Test substance

Test cigarettes: no storage of test cigarettes

Condensates: 5 ml of each condensate preparation transferred at day of mutagenicity assay into plastic tube with screw cap and stored at minus 80 degrees centigrade for at least 1 year

approx. 15 ml of each condensate preparation stored at plus 4 degrees centigrade for approx. 1 month

Proposal, records and evaluation sheets:

stored in our archives for at least 5 years, they can be claimed by the client

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6 RESULTS AND DISCUSSION

6.1 Text6.1.1 WSC-I of cigarettes6.1.1.1 Dry condensate, nicotine and water concentration and
puff number
(see TABLES 1 and 2)

Whole smoke condensates (WSC-I) were prepared 7 or 8 days prior to the mutagenicity assays (FIGURES A and B) using a glass impaction trap (FIGURE C). On each day of cigarette condensate preparation, condensates of the 2R1 standard reference cigarette were also prepared. The dry condensate nicotine and water concentration of WSC-I/DMSO suspension of the 2R1 standard reference cigarette were in the expected range.

6.1.1.2 Bacteriological examination of WSC-I

(see TABLE 3)

The diluted condensate suspensions were found to be free from bacteria growing on minimal-glucose agar.

6.1.2 Properties and responses of the tester strains

6.1.2.1 Properties

(see TABLE 4)

Cultures from frozen stock cultures of *Salmonella typhimurium* tester strains TA 98 and TA 100 were used for mutagenicity testing in this study. With tester strain TA 98 mutagens causing frame-

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shift mutation and with TA 100 mutagens causing base-pair substitution can be detected. The strains were checked for properties essential to the plate incorporation mutagenicity assay as recommended in the basic "method paper" by Ames et al. (1975):

- (1) auxotrophy in the form of histidine requirement,
- (2) the absence of the lipopolysaccharide barrier in the form of sensitivity to crystal violet,
- (3) the presence of the ampicillin resistance carrying extrachromosomal R factor and
- (4) the lack of an excision repair system (uvrB) in the form of sensitivity to UV light.

The bacteria responded in a manner characteristic for tester strains TA 98 and TA 100 as described by Ames et al. (1975).

6.1.2.2 Spontaneous reversion

(see TABLES 5 to 8 and FIGURES 1 and 2)

In order to determine the spontaneous reversion of the tester strains in the absence of cigarette smoke condensates, the solvent DMSO used for the condensates was mixed with top agar, tester strain bacteria and either S9 mix or S9 mix buffer and plated on minimal-glucose agar. The number of spontaneous revertants was determined automatically. Determinations were performed at the beginning and at the end of the mutagenicity assay on each day.

The number of spontaneous TA 98 revertants per plate was found to be 23.8 ± 1.4 (a) in the absence of S9 protein, when approx. 1.0×10^8 viable bacteria were added to each plate.

(a) MEAN \pm SE, N = 16

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The number of spontaneous TA 100 revertants per plate was found to be 121.3 ± 1.7 (a) in the absence of S9 protein, when approx. 1.0×10^8 viable bacteria were added to each plate.

The results of the spontaneous reversion are in agreement with our experience with the strains and findings by Ames et al. (1975) as well as by de Serres and Shelby (1979).

6.1.2.3 Mutagenicity of diagnostic mutagens (see TABLES 9 to 12 and FIGURES 3 and 4)

Positive and negative mutagenesis controls were included in this study using 5 diagnostic mutagens to confirm the reversion properties of the tester strains and the metabolic activity of the S9 protein used. The results of MMS, MNNG and 9-aminoacridine spot assays were in accordance with the results expected and published by Ames et al. (1975).

2-Aminoanthracene (2-AA) and 2-aminofluorene (2-AF) were tested in the plate incorporation mutagenicity assay at a single dose in the presence of S9 protein. The number of revertants per plate was nearly the same in both assays. These results are in accordance with those published by Zeiger et al. (1979) and Simmon (1979) and with those expected for TA 98 and TA 100.

In addition, an almost linear dose-response curve was obtained with 2-AF between 0 and 3 micrograms per plate in 2 plate incorporation mutagenicity assays. The mean specific mutagenicity was 124.5 revertants per microgram 2-AF with TA 98 and 48.5 with TA 100. These results were in agreement with previous studies.

(a) MEAN \pm SE, N = 16

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6.1.3 Properties of S9 protein

(see TABLES 13 to 15 and FIGURE 5)

The S9 protein was prepared from liver homogenate of Aroclor 1254-induced rats. Analytical data of the preparation used in this study are shown in TABLE 15. The specific AHH activity was 121.6 U/mg protein in the expected range.

The activity of the S9 protein used in this study for the biotransformation of promutagens to mutagens in the plate incorporation assay was determined with B(a)P, WSC-I of cigarette 2R1 and 2 diagnostic mutagens. The amount of 0.9 to 1.9 milligrams S9 protein per plate was found to be suitable for the plate incorporation mutagenicity assay of WSC-I using tester strains TA 98. The response of tester strain TA 98 to B(a)P and WSC-I of cigarette 2R1 metabolically activated by the S9 protein was in the expected range.

During the preparation of S9 mix, the S9 protein was diluted to 4 g/l and the S9 mix was sterile-filtered. During this filtration, the protein concentration was reduced to approx. 3.1 g/l S9 mix. The final amount of S9 protein per mutagenicity assay plate was 1.6 milligrams.

6.1.4 Mutagenicity of cigarette smoke condensates

6.1.4.1 Experimental approach

(see TABLES 16 to 29 and FIGURES A and B)

WSC-I of cigarettes of project "LEAR" and 2R1 were assayed for their mutagenicity in the plate incorporation assay, using *Salmonella typhimurium* tester strains TA 98 and TA 100 with metabolic activation by S9 protein from Aroclor 1254-induced rat livers.

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Condensates of each cigarette were assayed in 2 independent assays on 2 different days. All WSC-I were diluted to a final concentration of 2.5 milligrams dry condensate per milliliter and were assayed in the presence of S9 protein at 4 different condensate doses using 2 plates per dose. The number of revertants were counted automatically.

6.1.4.2 Dose response

(see FIGURES 6 to 19)

All condensates were assayed at 4 doses: 0, 0.05, 0.10 and 0.15 milligrams dry condensate/plate. Depending on the dose an approx. linear increase in the number of revertants was observed with condensates of all cigarettes.

6.1.4.3 Reversion assay

(see TABLES 30 and 31)

In order to test, whether colonies grown on minimal-glucose agar plates with limited histidine supply are revertants (a), individual colonies after 2-day growth on plates, to which the high dose of condensates had been added, were tested for histidine prototrophy on agar plates without histidine.

140 out of 140 (100 percent) colonies of tester strain TA 98 and 140 out of 140 (100 percent) colonies of tester strain TA 100 from mutagenicity test plates were found to be histidine prototrophs (revertants) in the subsequent reversion assay on plates without histidine.

(a) A trace amount of histidine was added to the top agar in the plate incorporation assay to allow the bacteria on the plate to undergo several divisions which are in many cases necessary for mutagenesis to occur. In case of massive cell death during exposure of the bacteria to a test substance more histidine is available to the individual surviving bacteria, and they undergo more cell divisions forming small colonies ("false revertants") which can be mistaken for revertants (Ames et al. (1975)).

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6.1.4.4 Specific mutagenicity

(see TABLES 32 to 51, FIGURES 2o and 21)

The mutagenic activity was expressed as the specific mutagenicity, defined as the regression coefficient "a" of the linear dose-response curve $y = ax + b$. The specific mutagenicity was calculated as the extrapolated increase in the number of revertants per milligram of dry condensate.

Using tester strain TA 98 to detect frameshift mutations, the specific mutagenicity of each cigarette obtained from assay 1 was statistically not different to that of assay 2. The specific mutagenicity was 2235 revertants per milligram dry condensate for LEAR o-17-2, 35o8 for LEAR o-17-3, 193o for LEAR 2-17-2, 2942 for LEAR 2-17-3, 2o23 for LEAR 4-17-2, 2988 for LEAR 4-17-3 and 1536 for 2R1. The cigarettes with the last digit number "2" were found to be statistically significantly less mutagenic than the corresponding cigarettes with the last digit number "3" (a). The cigarettes of project "LEAR" were statistically significantly higher mutagenic than 2R1.

Using tester strain TA 100 to detect base-pair substitution, the specific mutagenicity obtained in assay 1 was statistically not different to that in assay 2 for each cigarette. The specific mutagenicity was 859 revertants per milligram dry condensate for LEAR o-17-2, 1354 for LEAR o-17-3, 897 for LEAR 2-17-2, 1185 for LEAR 2-17-3, 863 for LEAR 4-17-2, 1000 for LEAR 4-17-3 and 696 for 2R1. The cigarettes LEAR o-17-2 and LEAR 2-17-2 were found to be statistically significantly less mutagenic, whereas the cigarette LEAR 4-17-2 was only numerically less mutagenic than the corresponding cigarettes with the last digit number "3" (a). The cigarettes of project "LEAR" were statistically significantly higher mutagenic than 2R1.

(a) LEAR cigarettes with the last digit number "2" differed from the corresponding cigarettes in higher amount of reducing sugars of filler (see TABLE B, PAGE 3-4).

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6.1.4.5 Relative specific mutagenicity
(see TABLES 48 and 49, FIGURE 22)

In order to compensate variations from study to study in the condensate preparation and mutagenicity assay performance, condensates of the standard reference cigarette 2R1 were used as an internal standard. These standard condensates were prepared on the same day and stored and assayed under the same conditions as the other cigarette condensates. The specific mutagenicity of the cigarettes were compared to that of the standard cigarette 2R1 which was set to 100 percent.

In case of tester strain ta 98 the relative specific mutagenicity was 146 percent for LEAR 0-17-2, 228 for LEAR 0-17-3, 126 for LEAR 2-17-2, 192 for LEAR 2-17-3, 132 for LEAR 4-17-2 and 195 for LEAR 4-17-3.

In case of tester strain TA 100 the relative specific mutagenicity was 123 percent for LEAR 0-17-2, 195 for LEAR 0-17-3, 129 for LEAR 2-17-2, 170 for LEAR 2-17-3, 124 for LEAR 4-17-2 and 144 for LEAR 4-17-3.

6.1.5 Conclusion

The specific mutagenicities of the condensates tested show a range from the highest value of LEAR 0-17-3 to the lowest value of 2R1 with a factor of 2.3 for frameshift mutation and 1.9 for base-pair substitution.

For frameshift mutation as well as for base-pair substitution the condensates of cigarettes with the last digit number "2" are less mutagenic than the corresponding cigarettes with the last digit number "3". The condensates of all cigarettes of project "LEAR" are more mutagenic than that of standard reference cigarette 2R1.

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6.2 Tables and Figures

| CIGARETTE | DATE OF CON-DENSATE PRE-PARATION | BATCH NO. | DRY CON-DENSATE CONC. (g/1) | NICOTINE CONC. (g/1) | WATER CONC. (g/1) | NUMBER OF PUFFS |
|-------------|----------------------------------|-----------|--------------------------------|-------------------------|----------------------|-----------------|
| 2R1 | 2.Nov.81 | 54 | 45.70 | 3.37 | 6.90 | 11.3 |
| | | 55 | 46.99 | 3.44 | 6.25 | 11.1 |
| | 17.Nov.81 | 56 | 50.82 | 3.85 | 6.88 | 11.5 |
| | | 57 | 47.33 | 3.65 | 5.52 | 11.2 |
| LEAR 0-17-2 | 2.Nov.81 | 01 | 19.67 | 1.62 | 5.29 | 8.1 |
| | | 02 | 20.00 | 1.63 | 5.02 | 7.2 |
| | 17.Nov.81 | 03 | 15.88 | 1.49 | 2.37 | 8.7 |
| | | 04 | 15.99 | 1.55 | 2.24 | 8.5 |
| LEAR 0-17-3 | 2.Nov.81 | 01 | 15.69 | 1.95 | 2.94 | 8.0 |
| | | 02 | 17.19 | 2.24 | 4.52 | 8.7 |
| | 17.Nov.81 | 03 | 16.51 | 2.08 | 2.82 | 8.8 |
| | | 04 | 16.59 | 2.07 | 2.50 | 8.8 |
| LEAR 2-17-2 | 2.Nov.81 | 01 | 19.42 | 1.43 | 4.09 | 8.8 |
| | | 02 | 17.64 | 1.46 | 4.95 | 9.0 |
| | 17.Nov.81 | 03 | 17.47 | 1.41 | 2.89 | 8.2 |
| | | 04 | 16.31 | 1.31 | 1.85 | 8.4 |
| LEAR 2-17-3 | 2.Nov.81 | 01 | 19.73 | 1.60 | 4.40 | 9.1 |
| | | 02 | 12.44 | 1.21 | 3.28 | 9.0 |
| | 17.Nov.81 | 03 | 19.64 | 1.51 | 2.11 | 8.9 |
| | | 04 | 16.95 | 1.45 | 1.59 | 9.0 |
| LEAR 4-17-2 | 2.Nov.81 | 01 | 17.36 | 1.50 | 3.53 | 9.0 |
| | | 02 | 19.18 | 1.66 | 5.26 | 8.8 |
| | 17.Nov.81 | 03 | 18.34 | 1.50 | 2.18 | 8.7 |
| | | 04 | 18.72 | 1.64 | 2.53 | 8.4 |
| LEAR 4-17-3 | 2.Nov.81 | 01 | 18.31 | 1.83 | 4.64 | 8.2 |
| | | 02 | 17.75 | 1.91 | 4.27 | 8.2 |
| | 17.Nov.81 | 03 | 18.44 | 1.88 | 2.01 | 8.5 |
| | | 04 | 17.86 | 1.86 | 1.79 | 8.5 |

TABLE 1

CONCENTRATION OF DRY CONDENSATE, NICOTINE AND WATER OF WSC-I/DMSO SUSPENSION
AND PUFF NUMBER OF CIGARETTES

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| CIGARETTE | DRY CONDENSATE | | NICOTINE | | WATER | | NUMBER OF | |
|-------------|----------------|-------|----------|-------|-------|-------|-----------|----------|
| | CONC. | (g/l) | CONC. | (g/l) | CONC. | (g/l) | PUFFS | (1/cig.) |
| | MEAN | SE | MEAN | SE | MEAN | SE | MEAN | SE |
| 2R1 | 55.50 | 0.65 | 3.48 | 0.11 | 6.39 | 0.33 | 11.3 | 0.1 |
| LEAR 0-17-2 | 17.89 | 1.13 | 1.57 | 0.03 | 3.73 | 0.82 | 8.1 | 0.3 |
| LEAR 0-17-3 | 16.50 | 0.31 | 2.09 | 0.06 | 3.20 | 0.45 | 8.6 | 0.2 |
| LEAR 2-17-2 | 17.71 | 0.64 | 1.40 | 0.03 | 3.45 | 0.68 | 8.6 | 0.2 |
| LEAR 2-17-3 | 17.19 | 1.71 | 1.44 | 0.08 | 2.85 | 0.63 | 9.00 | 0.0 |
| LEAR 4-17-2 | 18.40 | 0.39 | 1.58 | 0.04 | 3.38 | 0.69 | 8.73 | 0.1 |
| LEAR 4-17-3 | 18.09 | 0.17 | 1.87 | 0.02 | 3.18 | 0.74 | 8.35 | 0.1 |

TABLE 2

MEAN CONCENTRATION OF DRY CONDENSATE, NICOTINE AND WATER OF WSC-I/DMSO SUSPENSION
AND MEAN PUFF NUMBER OF CIGARETTES, N = 4

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| DATE | CIGARETTE | CONDENSATE BATCH NO. | BACTERIAL CONTAMINATION (CFU/o.15 mg dry cond.) | |
|-----------|-------------|-------------------------|--|---|
| | | | PLATE 1 | 2 |
| 9.Nov.81 | 2R1 | 54 | o | o |
| | LEAR o-17-2 | o1 | o | o |
| | LEAR o-17-3 | o1 | o | o |
| | LEAR 2-17-2 | o1 | o | o |
| | LEAR 2-17-3 | o1 | o | o |
| | LEAR 4-17-2 | o1 | o | o |
| | LEAR 4-17-3 | o1 | o | o |
| 10.Nov.81 | 2R1 | 54 | o | o |
| | LEAR o-17-2 | o1 | o | o |
| | LEAR o-17-3 | o1 | o | o |
| | LEAR 2-17-2 | o1 | o | o |
| | LEAR 2-17-3 | o1 | o | o |
| | LEAR 4-17-2 | o1 | o | o |
| | LEAR 4-17-3 | o1 | o | o |
| 24.Nov.81 | 2R1 | 56 | o | o |
| | LEAR o-17-2 | o3 | o | o |
| | LEAR o-17-3 | o3 | o | o |
| | LEAR 2-17-2 | o3 | o | o |
| | LEAR 2-17-3 | o3 | o | o |
| | LEAR 4-17-2 | o3 | o | o |
| | LEAR 4-17-3 | o3 | o | o |
| 25.Nov.81 | 2R1 | 56 | o | o |
| | LEAR o-17-2 | o3 | o | o |
| | LEAR o-17-3 | o3 | o | o |
| | LEAR 2-17-2 | o3 | o | o |
| | LEAR 2-17-3 | o3 | o | o |
| | LEAR 4-17-2 | o3 | o | o |
| | LEAR 4-17-3 | o3 | o | o |

TABLE 3

BACTERIOLOGICAL EXAMINATION OF WSC-I OF CIGARETTES

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| PARAMETER | TESTER STRAIN | |
|----------------------------|---------------|--------|
| | TA 98 | TA 100 |
| histidine requirement | | |
| growth without histidine | o | o |
| growth with histidine | + | + |
| crystal violet sensitivity | + | + |
| ampicillin resistance | + | + |
| uv sensitivity | + | + |

TABLE 4

PROPERTIES OF THE TESTER STRAINS TA 98 AND TA 100

dates of determinations: 6.Oct.81
30.Nov.81

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| DATE | TEST NO. | ABSORB- ANCE (CFU/plate) | NUMBER OF VIABLE BACTERIA PLATED IN THE MUTAGENICITY ASSAY (bacteria/plate) | | | | RSD (%) | |
|------------------------------|-------------|--------------------------------|---|-----|-----|-----|-------------------------|------|
| | | | PLATE | | | | | |
| | | | 1 | 2 | 3 | 4 | | |
| 9.Nov.81 | 1 | 1.78 | 158 | 105 | 126 | 166 | 138.8 | 20.5 |
| | 2 | 1.55 | 81 | 67 | 49 | 107 | 76.0 | 32.2 |
| 24.Nov.81 | 1 | 1.75 | 121 | 146 | 101 | 160 | 132.0 | 19.9 |
| | 2 | 1.60 | 49 | 99 | 42 | 80 | 67.5 | 39.6 |
| 9.Nov.81 and 24.Nov.81 | 1 and 2 | - | - | - | - | - | <u>103.6 ± 10.2</u> (a) | 39.3 |

TABLE 5

ABSORBANCE, VIABILITY AND NUMBER OF VIABLE TA 98 BACTERIA PLATED IN THE MUTAGENICITY ASSAY

After growth for 12 h, suspension cultures were diluted 10E6-fold and 0.1 mL of the dilution plated onto minimal-glucose agar plates with a sufficient amount of histidine (10 µmol/plate). Tests were performed at the beginning (test no. 1) and at the end (test no. 2) of each plate incorporation assay. Plates were counted automatically.

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(a) MEAN ± SE, N = 16

| DATE | TEST NO. | SPONTANEOUS REVERTANTS (number of revertants/ plate) | | | | STATISTICAL PARAMETER | |
|------------------------------|-------------|--|----|----|----|-----------------------|------|
| | | PLATE | | | | MEAN | RSD |
| | | 1 | 2 | 3 | 4 | | |
| 9.Nov.81 | 1 | 24 | 22 | 22 | 30 | 24.5 | 15.5 |
| | 2 | 14 | 19 | 19 | 23 | 18.8 | 19.7 |
| 24.Nov.81 | 1 | 28 | 22 | 30 | 33 | 28.3 | 16.4 |
| | 2 | 27 | 14 | 25 | 28 | 23.5 | 27.5 |
| 9.Nov.81 and 24.Nov.81 | 1 and 2 | - | - | - | - | 23.8 <u>±</u> 1.4 (a) | 23.2 |

TABLE 6

SPONTANEOUS REVERTANTS OF STRAIN TA 98 IN THE ABSENCE OF S9 PROTEIN

Test performed at the beginning (test no. 1) and at the end (test no. 2) of each plate incorporation assay. Plates were counted automatically.

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(a) MEAN ± SE, N = 16

| DATE | TEST NO. | ABSOR- BANCE | VIABILITY (CFU/plate) | NUMBER OF VIABLE BACTERIA PLATED IN THE MUTAGENICITY ASSAY (bacteria/plate) | | | | |
|-------------------------------|-------------|-----------------|--------------------------|---|-----|-----|-----------------------|-------------------------|
| | | | | PLATE | | | | MEAN (10 ⁶) |
| | | | | 1 | 2 | 3 | 4 | |
| 10.Nov.81 | 1 | 1.75 | 71 | 77 | 85 | 86 | 79.8 | 8.9 |
| | 2 | 1.60 | 50 | 41 | 80 | - | 57.0 | 35.8 |
| 25.Nov.81 | 1 | 1.70 | 123 | 120 | 140 | 144 | 131.8 | 9.1 |
| | 2 | 1.60 | 100 | 116 | 118 | 114 | 112.0 | 7.3 |
| 10.Nov.81 and 25.Nov.81 | 1 and 2 | - | - | - | - | - | 97.7 <u>±</u> 8.0 (a) | 31.6 |

TABLE 7

ABSORBANCE, VIABILITY AND NUMBER OF VIABLE TA 100 BACTERIA PLATED IN THE MUTAGENICITY ASSAY

After growth for 12 h, suspension cultures were diluted 10⁶-fold and 0.1 ml of the dilution plated onto minimal-glucose agar plates with a sufficient amount of histidine (10 µmol/plate). Tests were performed at the beginning (test no. 1) and at the end (test no. 2) of each plate incorporation assay. Plates were counted automatically.

(a) MEAN ± SE, N = 16

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| DATE | TEST NO. | SPONTANEOUS REVERTANTS (number of revertants/ plate) | | | | STATISTICAL PARAMETER | |
|-------------------------------|-------------|--|-----|-----|-----|-----------------------|------|
| | | PLATE | | | | MEAN | RSD |
| | | 1 | 2 | 3 | 4 | | |
| 10.Nov.81 | 1 | 131 | 121 | 124 | 120 | 124.0 | 4.0 |
| | 2 | 131 | 115 | 121 | 103 | 117.5 | 10.0 |
| 25.Nov.81 | 1 | 121 | 117 | 131 | 124 | 123.3 | 4.8 |
| | 2 | 117 | 120 | 120 | 125 | 120.5 | 2.8 |
| 10.Nov.81 and 25.Nov.81 | 1 and 2 | - | - | - | - | 121.3 \pm 1.7 (a) | 5.7 |

TABLE 8

SPONTANEOUS REVERTANTS OF STRAIN TA 100 IN THE ABSENCE OF S9 PROTEIN

Test performed at the beginning (test no. 1) and at the end (test no. 2) of each plate incorporation assay. Plates were counted automatically.

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(a) MEAN \pm SE, N = 16

| DATE | MUTAGENICITY (mean number of revertants/plate) | | | | |
|------------------------|--|--------------------------|--------------------------|--------------------------------|---------------------------|
| | MMS | MNNG | 9-AMINO- ACRIDINE | 2-AA + S9 PROT. (a) | 2-AF + S9 PROT. (a) |
| DOSE (mg/plate) | | | | | |
| | 2.8 | 0.002 | 0.010 | 0.002 | 0.002 |
| 9.Nov.81 | 2 | 9 | 0 | 1502 | 227 |
| 24.Nov.81 | 4 | 3 | 0 | 1439 | 250 |
| <hr/> | | | | | |
| SUMMARIZED RESPONSE | 0 | 0 | 0 | +++ | ++ |
| PUBLISHED RESPONSE | 0,+ | 0 | 0 | +++ | +++ |
| REFERENCE | Ames et al. (1975) | Ames et al. (1975) | Ames et al. (1975) | Zeiger et al. (1979) (b) | Simmon (1979) (c) |

TABLE 9

MUTAGENICITY OF DIAGNOSTIC MUTAGENS TOWARDS STRAIN TA 98

When not specified, tests were performed in the absence of S9 protein, the mean of 2 plates/diagnostic mutagen. 2-AA and 2-AF in the presence of S9 protein were dissolved in top agar, all other substances were tested in spot assays.

o: .LT.20 revertants/plate

+: 20 to 100 revertants/plate

++: 100 to 400 revertants/plate

+++: GT, 400 revertants/plate

+, o: variable response, .LT. 20 to 100 revertants/plate

Number of colonies was corrected for spontaneous revertants.

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(a) 1.5 to 1.6 mg S9 protein/plate

(b) 2.35 mg S9 protein/plate

(c) 1 to 2 mg S9 protein/plate

| DATE | MUTAGENICITY (mean number of revertants/plate) | | | | |
|---------------------|--|--------------------|--------------------|------------------------|------------------------|
| | MMS | MNNG | 9-AMINO-ACRIDINE | 2-AA + S9 PROT. (a) | 2-AF + S9 PROT. (a) |
| DOSE (mg/plate) | | | | | |
| | 2.8 | 0.002 | 0.010 | 0.002 | 0.002 |
| 10.Nov.81 | 600 | 320 | 11 | 1780 | 88 |
| 24.Nov.81 | 536 | 338 | 0 | 1650 | 112 |
| SUMMARIZED RESPONSE | | | | | |
| +++ | ++ | 0 | +++ | ++ | |
| PUBLISHED RESPONSE | | | | | |
| +++ | ++ | 0 | - | +++ | |
| REFERENCE | Ames et al. (1975) | Ames et al. (1975) | Ames et al. (1975) | - | Simmon (1979) (b) |

TABLE 10

MUTAGENICITY OF DIAGNOSTIC MUTAGENS TOWARDS STRAIN TA 100

When not specified, tests were performed in the absence of S9 protein, the mean of 2 plates/diagnostic mutagen. 2-AA and 2-AF in the presence of S9 protein were dissolved in top agar, all other substances were tested in spot assays.

0: <LT.20 revertants/plate

+: 20 to 100 revertants/plate

++: 100 to 400 revertants/plate

+++: >400 revertants/plate

+, 0: variable response, <LT.20 to 100 revertants/plate

Number of colonies was corrected for spontaneous revertants.

(a) 1.5 to 1.6 mg S9 protein/plate
(b) 1 to 2 mg S9 protein/plate

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| DATE | DOSE | MUTAGENICITY (number of revertants/ plate) | | | | MEAN | RSD | REGRESS. COEFF. | CORR. COEFF. | | | | |
|------------------------------|------|--|-----|-----|-----|-------|------|--------------------|-----------------|--|--|--|--|
| | | PLATE | | | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | | | | | | | | |
| | | (ug/ plate) | | | | (o/o) | | (1/ug) | | | | | |
| 9.Nov.81 | 0 | 35 | 29 | 37 | 40 | 35.3 | 13.2 | | | | | | |
| | 1 | 135 | 106 | 114 | 92 | 111.8 | 16.1 | | | | | | |
| | 2 | 222 | 271 | 269 | 288 | 262.5 | 10.8 | | | | | | |
| | 3 | 387 | 355 | 403 | 369 | 378.5 | 5.5 | 118.0 | 0.993 | | | | |
| 24.Nov.81 | 0 | 32 | 31 | 30 | 23 | 29.0 | 14.1 | | | | | | |
| | 1 | 117 | 123 | 154 | 116 | 127.5 | 14.1 | | | | | | |
| | 2 | 271 | 254 | 331 | 261 | 279.3 | 12.6 | | | | | | |
| | 3 | 417 | 397 | 443 | 404 | 415.3 | 4.9 | 131.1 | 0.997 | | | | |
| 9.Nov.81 and 24.Nov.81 | - | - | - | - | - | - | - | 124.5 (a) | 0.992 | | | | |

TABLE 11

MUTAGENICITY OF 2-AMINOFLUORENE TOWARDS STRAIN TA 98
IN THE PRESENCE OF S9 PROTEIN

(a) MEAN

2026048959

| DATE | DOSE (ug/ plate) | MUTAGENICITY (number of revertants/ plate) | | | | MEAN | RSD | REGRESS. COEFF. | CORR. COEFF. | | | | |
|------------------------------|------------------------|--|-----|-----|-----|-------|------|--------------------|-----------------|--|--|--|--|
| | | PLATE | | | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | | | | | | | | |
| 10.Nov.81 | 0 | 121 | 98 | 107 | 124 | 112.5 | 10.8 | | | | | | |
| | 1 | 158 | 177 | 150 | 164 | 162.3 | 7.0 | | | | | | |
| | 2 | 185 | 207 | 201 | 210 | 200.8 | 5.6 | | | | | | |
| | 3 | 249 | 282 | 255 | 258 | 261.0 | 5.6 | 48.4 | 0.997 | | | | |
| 25.Nov.81 | 0 | 102 | 120 | 123 | - | 117.3 | 8.8 | | | | | | |
| | 1 | 182 | 185 | 168 | 196 | 182.8 | 6.3 | | | | | | |
| | 2 | 194 | 245 | 227 | 252 | 229.5 | 11.3 | | | | | | |
| | 3 | 271 | 246 | 265 | 274 | 264.0 | 4.8 | 48.7 | 0.990 | | | | |
| 9.Nov.81 and 24.Nov.81 | - | - | - | - | - | - | - | 48.5 (a) | 0.992 | | | | |

TABLE 12

MUTAGENICITY OF 2-AMINOFLUORENE TOWARDS STRAIN TA 100
IN THE PRESENCE OF S9 PROTEIN

(a) MEAN

2026048960

| PARAMETER | UNIT | S9 FRACTION |
|--------------------------------|--------------|-------------|
| batch no. | - | 81.A |
| number of rats | - | 19 |
| date of Aroclor administration | - | 8.Aug.81 |
| date of sacrifice | - | 13.Aug.81 |
| body weight | g | 220.0 |
| liver weight | g | 10.3 |
| date of S9 preparation | - | 13.Aug.81 |
| protein concentration | g/l | 42.8 |
| specific AHH activity | U/mg protein | 121.6 |
| bacterial contamination (a) | CFU/ml | 18 |

TABLE 13

ANALYTICAL DATA OF S9 PROTEIN, BATCH NO. 81.A

(a) Results of unfiltered S9 fraction. S9 protein added to the other components of the S9 mix and sterile-filtered prior to use in the plate incorporation assay.

2026048961

| TEST SUBSTANCE | S9 PROTEIN (mg/plate) | MUTAGENICITY (number of revertants/ plate) | | | | STATISTICAL PARAMETER | |
|-------------------|--------------------------|--|------|------|------|-----------------------|------------|
| | | PLATE | | | | MEAN | RSD (%) |
| | | 1 | 2 | 3 | 4 | | |
| DMSO | 0 | 26 | 19 | 15 | 30 | 22.5 | 30.0 |
| | 0.46 | 24 | 18 | 27 | 28 | 24.3 | 18.6 |
| | 0.92 | 28 | 19 | 16 | 34 | 24.3 | 34.1 |
| | 1.85 | 20 | 22 | 20 | 26 | 22.0 | 12.9 |
| | 3.47 | 27 | 43 | 30 | 36 | 34.0 | 20.8 |
| | 4.62 | 33 | 29 | 35 | 36 | 33.3 | 9.3 |
| 2R1 | 0 | 17 | 23 | 26 | 24 | 22.5 | 17.2 |
| | 0.46 | 69 | 75 | 89 | 79 | 78.0 | 10.8 |
| | 0.92 | 231 | 165 | 181 | 206 | 195.8 | 14.8 |
| | 1.85 | 185 | 198 | 233 | 218 | 208.5 | 10.2 |
| | 3.47 | 73 | 163 | 155 | 179 | 142.5 | 33.3 |
| | 4.62 | 116 | 82 | 84 | 90 | 93.0 | 16.9 |
| B(a)P | 0 | 26 | 21 | 28 | 25 | 25.0 | 11.8 |
| | 0.46 | 111 | 126 | 138 | 107 | 120.5 | 11.8 |
| | 0.92 | 246 | 251 | 219 | 295 | 252.8 | 12.5 |
| | 1.85 | 474 | 495 | 545 | - | 504.7 | 7.2 |
| | 3.47 | 742 | 648 | 665 | 672 | 681.8 | 6.1 |
| | 4.62 | 571 | 591 | 567 | 516 | 561.3 | 5.7 |
| 2-AA | 0 | 31 | 16 | 27 | 20 | 23.5 | 28.8 |
| | 0.46 | 2632 | 2379 | 2622 | 2445 | 2519.5 | 5.0 |
| | 0.92 | 2330 | 2487 | 2351 | 2289 | 2364.3 | 3.6 |
| | 1.85 | 1339 | 1294 | 1264 | 1168 | 1258.8 | 5.6 |
| | 3.47 | 747 | 763 | 773 | 537 | 705.0 | 16.0 |
| | 4.62 | 526 | 606 | 568 | 606 | 576.5 | 6.6 |
| 2-AF | 0 | 20 | 24 | 26 | 19 | 22.3 | 14.9 |
| | 0.46 | 691 | 460 | - | 662 | 604.3 | 20.8 |
| | 0.92 | 426 | 461 | 458 | 490 | 458.8 | 5.7 |
| | 1.85 | 269 | 245 | 258 | 242 | 253.5 | 4.9 |
| | 3.47 | 154 | 183 | 188 | 180 | 176.3 | 8.6 |
| | 4.62 | 141 | 129 | 133 | 103 | 126.5 | 13.0 |

TABLE 14

PROMUTAGEN ACTIVATION BY VARIOUS DOSES OF S9 PROTEIN, BATCH NO. 81.A,
STRAIN TA 98

Test substances: DMSO (40 µl/plate), WSC-I (0.1 mg/plate), B(a)P (5 µg/plate),
2-AA (2 µg/plate) and 2-AF (2 µg/plate). The test substances were applied
together with 40 µl DMSO.

date of determination: 2.Sep.81

2026048962

| DATE | PROTEIN CONCENTRATION | | PROTEIN AMOUNT (a) (mg/plate) | SPECIFIC AHM ACTIVITY (U/mg protein) | BACTERIAL CONTAMINATION (CFU/ml) |
|--------------------------|-----------------------|-------------------|----------------------------------|---|-------------------------------------|
| | UNFILTERED (g/l) | FILTERED (g/l) | | | |
| | | | | | |
| 9.Nov.81 | 4.0 | 3.1 | 1.5 | 133.7 | 0 |
| 10.Nov.81 | 4.0 | 3.1 | 1.6 | 130.0 | 0 |
| 24.Nov.81 | 4.0 | 3.1 | 1.6 | 156.3 | 0 |
| 25.Nov.81 | 4.0 | 2.9 | 1.5 | 119.7 | 0 |
| 9.Nov.81 to 25.Nov.81 | - | - | - | 134.9 <u>±</u> 7.7 (a) | - |

TABLE 15

ANALYTICAL DATA OF S9 MIXES

S9 mixes were stored at minus 80 degrees centigrade until determination.

date of determination: 26.Nov.81

(a) MEAN ± SE, N = 4

2026048963

A-/2059.00

| DATE | CON- DEN- SATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|-----------|-------------------------------|------|--------------------------------|------|------------|------|---------------------------|------------|
| | | | DIL.SUSP.1 | | DIL.SUSP.2 | | MEAN | RSD (%) |
| | | | PLATE 1.1 (MG/ PLATE) | 1.2 | 2.1 | 2.2 | | |
| 9.NOV.81 | 54 | 0.00 | 45. | 38. | 29. | 30. | 35.5 | 21.1 |
| | | 0.05 | 100. | 100. | 96. | 92. | 97.0 | 3.9 |
| | | 0.10 | 173. | 182. | 193. | 189. | 184.2 | 4.8 |
| | | 0.15 | 258. | 283. | 260. | 260. | 265.2 | 4.5 |
| | 55 | 0.00 | 23. | 38. | 26. | 27. | 28.5 | 23.0 |
| | | 0.05 | 103. | 108. | 96. | 108. | 103.7 | 5.5 |
| | | 0.10 | 207. | 194. | 194. | 214. | 202.2 | 4.9 |
| | | 0.15 | 271. | 259. | 222. | 246. | 249.5 | 8.4 |
| 24.NOV.81 | 56 | 0.00 | 28. | 28. | 30. | 30. | 29.0 | 4.0 |
| | | 0.05 | 95. | 109. | 78. | 113. | 98.7 | 16.0 |
| | | 0.10 | 171. | 172. | 172. | 186. | 175.2 | 4.1 |
| | | 0.15 | 265. | 258. | 263. | 251. | 259.2 | 2.4 |
| | 57 | 0.00 | 24. | 28. | 34. | 28. | 28.5 | 14.5 |
| | | 0.05 | 96. | 122. | 103. | 78. | 99.7 | 18.2 |
| | | 0.10 | 170. | 167. | 182. | 200. | 179.7 | 8.3 |
| | | 0.15 | 245. | 259. | 266. | 259. | 257.2 | 3.4 |

TABLE 16

MUTAGENICITY OF WSC-1 OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA 98

2026048964

502.00/ T501 /2058.00

A-/2059.00

| DATE | CON- DEN- SATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|-----------|-------------------------------|------|--------------------------------|------|--------------|------|---------------------------|------------|
| | | | DIL. SUSP. 1 | | DIL. SUSP. 2 | | MEAN | RSD (%) |
| | | | PLATE 1.1 (MG/ PLATE) | 1.2 | 2.1 | 2.2 | | |
| 9.NOV.81 | 1 | 0.00 | 26. | 29. | 27. | 31. | 28.2 | 7.8 |
| | | 0.05 | 124. | 95. | 121. | 120. | 115.0 | 11.7 |
| | | 0.10 | 218. | 206. | 216. | 214. | 213.5 | 2.5 |
| | | 0.15 | 306. | 329. | 329. | 313. | 319.2 | 3.6 |
| | 2 | 0.00 | 21. | 29. | 58. | 30. | 34.5 | 46.9 |
| | | 0.05 | 84. | 110. | 93. | 100. | 96.7 | 11.4 |
| | | 0.10 | 199. | 206. | 221. | 217. | 210.7 | 4.8 |
| | | 0.15 | 320. | 332. | 347. | 313. | 328.0 | 4.5 |
| 24.NOV.81 | 3 | 0.00 | 35. | 44. | 28. | 19. | 31.5 | 33.6 |
| | | 0.05 | 135. | 112. | 119. | 101. | 116.7 | 12.2 |
| | | 0.10 | 289. | 231. | 261. | 245. | 256.5 | 9.7 |
| | | 0.15 | 401. | 407. | 396. | 354. | 389.5 | 6.2 |
| | 4 | 0.00 | 33. | 29. | 37. | 37. | 34.0 | 11.3 |
| | | 0.05 | 127. | 105. | 131. | 126. | 122.2 | 9.6 |
| | | 0.10 | 310. | 286. | 288. | 242. | 281.5 | 10.1 |
| | | 0.15 | 393. | 387. | 453. | 412. | 411.2 | 7.2 |

TABLE 17

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION, STRAIN TA 98

2026048965

502.00/ T501 /2059.00

A-72059.00

| DATE | CON- DEN- SATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|-----------|-------------------------------|----------------|----------------------|------|--------------|------|---------------------------|------------|
| | | | DIL. SUSP. 1 | | DIL. SUSP. 2 | | MEAN | RSD (%) |
| | | | PLATE 1.1 | 1.2 | 2.1 | 2.2 | | |
| | | (MG/ PLATE) | | | | | | |
| 9.NOV.81 | 1 | 0.00 | 23. | 25. | 22. | 25. | 23.7 | 6.3 |
| | | 0.05 | 163. | 153. | 106. | 122. | 136.0 | 19.5 |
| | | 0.10 | 311. | 406. | 333. | 356. | 351.5 | 11.6 |
| | | 0.15 | 463. | 479. | 516. | 499. | 489.2 | 4.7 |
| | 2 | 0.00 | 28. | 37. | 32. | 42. | 34.7 | 17.5 |
| | | 0.05 | 159. | 115. | 162. | 178. | 153.5 | 17.6 |
| | | 0.10 | 363. | 302. | 386. | 386. | 359.2 | 11.0 |
| | | 0.15 | 432. | 625. | 577. | 553. | 546.7 | 15.0 |
| 24.NOV.81 | 3 | 0.00 | 20. | 33. | 27. | 26. | 26.5 | 20.1 |
| | | 0.05 | 188. | 177. | 207. | 181. | 188.2 | 7.1 |
| | | 0.10 | 436. | 404. | 415. | 376. | 407.7 | 6.1 |
| | | 0.15 | 576. | 556. | 673. | 386. | 547.7 | 21.8 |
| | 4 | 0.00 | 40. | 24. | 36. | 24. | 31.0 | 26.6 |
| | | 0.05 | 190. | 177. | 168. | 158. | 173.2 | 7.8 |
| | | 0.10 | 400. | 392. | 414. | 407. | 403.2 | 2.3 |
| | | 0.15 | 602. | 547. | 593. | 581. | 580.7 | 4.1 |

TABLE 18:

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ T501 /2059.00

2026048966

A-/2059.00

| DATE | CON- DEN- SATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|----------|-------------------------------|------|--------------------------------|------|------------|------|---------------------------|------------|
| | | | DIL.SUSP.1 | | DIL.SUSP.2 | | MEAN | RSD (%) |
| | | | PLATE 1.1 (MG/ PLATE) | 1.2 | 2.1 | 2.2 | | |
| 9.NOV.81 | 1 | 0.00 | 25. | 33. | 36. | 38. | 33.0 | 17.3 |
| | | 0.05 | 81. | 82. | 85. | 92. | 85.0 | 5.8 |
| | | 0.10 | 167. | 182. | 161. | 194. | 176.5 | 8.2 |
| | | 0.15 | 287. | 254. | 279. | 286. | 276.5 | 5.6 |
| | 2 | 0.00 | 37. | 34. | 29. | 25. | 31.2 | 17.0 |
| | | 0.05 | 138. | 104. | 104. | 83. | 107.2 | 21.2 |
| | | 0.10 | 201. | 180. | 212. | 200. | 198.2 | 6.7 |
| | | 0.15 | 335. | 303. | 322. | 295. | 313.7 | 5.8 |
| | 3 | 0.00 | 25. | 32. | 26. | 27. | 27.5 | 14.3 |
| | | 0.05 | 111. | 117. | 137. | 109. | 118.5 | 10.8 |
| | | 0.10 | 256. | 225. | 229. | 168. | 219.5 | 16.5 |
| | | 0.15 | 390. | 364. | 350. | 341. | 361.2 | 5.9 |
| | 4 | 0.00 | 38. | 22. | 24. | 25. | 27.2 | 26.7 |
| | | 0.05 | 119. | 75. | 112. | 98. | 101.0 | 17.2 |
| | | 0.10 | 191. | 267. | 211. | 188. | 214.2 | 17.1 |
| | | 0.15 | 320. | 295. | 355. | 317. | 321.7 | 7.7 |

TABLE 19

MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 98

2026048967

502.00 / T501 /2059.00

A-2059.00

| DATE | CON- | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL | |
|-----------|------|------|----------------------|-------|------------|------------|-------------|------|
| | | | DEN- | | PARAMETERS | | | |
| | | | SATE | BATCH | DIL.SUSP.1 | DIL.SUSP.2 | PLATE | MEAN |
| | | | (MG/ | | | | | RSD |
| | | | PLATE | | | | (%) | |
| | | | (MG/ | | | | | |
| | | | PLATE) | | | | | |
| 9.NOV.81 | 1 | 0.00 | 33. | 26. | 21. | 25. | 26.2 | 19.0 |
| | | 0.05 | 147. | 149. | 176. | 143. | 153.7 | 9.8 |
| | | 0.10 | 245. | 257. | 291. | 300. | 273.2 | 9.7 |
| | | 0.15 | 358. | 433. | 418. | 448. | 414.2 | 9.5 |
| | 2 | 0.00 | 33. | 21. | 25. | 23. | 25.5 | 20.6 |
| | | 0.05 | 147. | 137. | 151. | 118. | 138.2 | 10.7 |
| | | 0.10 | 337. | 342. | 385. | 334. | 349.5 | 6.8 |
| | | 0.15 | 463. | 530. | 513. | 428. | 483.5 | 9.7 |
| 24.NOV.81 | 3 | 0.00 | 37. | 28. | 32. | 26. | 30.7 | 15.8 |
| | | 0.05 | 152. | 158. | 145. | 162. | 154.2 | 4.8 |
| | | 0.10 | 380. | 315. | 314. | 310. | 329.7 | 10.2 |
| | | 0.15 | 419. | 487. | 494. | 434. | 458.5 | 8.2 |
| | 4 | 0.00 | 43. | 24. | 30. | 30. | 31.7 | 25.2 |
| | | 0.05 | 177. | 156. | 180. | 171. | 171.0 | 5.2 |
| | | 0.10 | 339. | 305. | 353. | 376. | 343.2 | 8.7 |
| | | 0.15 | 490. | 460. | 516. | 508. | 493.5 | 5.0 |

TABLE 2o

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 98

2026048968

502.00/ T501. /2059.00

A-2059.00

| DATE | CON- DEN- STATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|----------|--------------------------------|------|--------------------------------|------|------------|------|---------------------------|------------|
| | | | DIL.SUSP.1 | | DIL.SUSP.2 | | MEAN | RSD (%) |
| | | | PLATE 1.1 (MG/ PLATE) | 1.2 | 2.1 | 2.2 | | |
| 9.NOV.81 | 1 | 0.00 | 35. | 23. | 28. | 18. | 26.0 | 27.9 |
| | | 0.05 | 97. | 89. | 113. | 111. | 102.5 | 11.2 |
| | | 0.10 | 211. | 241. | 258. | 213. | 230.7 | 9.9 |
| | | 0.15 | 308. | 280. | 315. | 349. | 313.0 | 9.1 |
| | 2 | 0.00 | 30. | 42. | 29. | 20. | 30.2 | 29.3 |
| | | 0.05 | 100. | 105. | 94. | 101. | 100.0 | 4.5 |
| | | 0.10 | 236. | 226. | 208. | 228. | 224.5 | 5.3 |
| | | 0.15 | 291. | 326. | 322. | 389. | 332.0 | 12.4 |
| | 3 | 0.00 | 27. | 28. | 28. | 32. | 28.7 | 7.7 |
| | | 0.05 | 114. | 126. | 109. | 113. | 115.5 | 6.3 |
| | | 0.10 | 215. | 215. | 298. | 219. | 236.7 | 17.3 |
| | | 0.15 | 389. | 362. | 328. | 358. | 359.2 | 7.0 |
| | 4 | 0.00 | 35. | 34. | 37. | 27. | 33.5 | 13.5 |
| | | 0.05 | 117. | 135. | 110. | 107. | 117.2 | 10.7 |
| | | 0.10 | 179. | 186. | 255. | 242. | 215.5 | 17.9 |
| | | 0.15 | 280. | 255. | 370. | 316. | 305.2 | 16.3 |

TABLE 21

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION, STRAIN TA 98

2026048969

502.00/ T501 /2059.00

A-/2059.00

| DATE | CON- DEN- SATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|-----------|-------------------------------|----------------|----------------------|------|------------|------|---------------------------|------------|
| | | | DIL.SUSP.1 | | DIL.SUSP.2 | | MEAN | RSD (%) |
| | | | PLATE 1.1 | 1.2 | 2.1 | 2.2 | | |
| | | (MG/ PLATE) | | | | | | |
| 9.NOV.81 | 1 | 0.00 | 27. | 28. | 21. | 28. | 26.0 | 12.9 |
| | | 0.05 | 151. | 72. | 118. | 89. | 107.5 | 32.2 |
| | | 0.10 | 322. | 289. | 306. | 280. | 299.2 | 6.2 |
| | | 0.15 | 501. | 506. | 437. | 485. | 482.2 | 6.5 |
| | 2 | 0.00 | 34. | 34. | 30. | 32. | 32.5 | 5.6 |
| | | 0.05 | - | - | 137. | 136. | 136.5 | 0.5 |
| | | 0.10 | 299. | 301. | 340. | 330. | 317.5 | 6.5 |
| | | 0.15 | 426. | 439. | 490. | 486. | 460.2 | 7.1 |
| 24.NOV.81 | 3 | 0.00 | 28. | 31. | 24. | 38. | 30.2 | 19.5 |
| | | 0.05 | 178. | 119. | 158. | 129. | 146.2 | 18.6 |
| | | 0.10 | 356. | 284. | 325. | 303. | 317.0 | 9.8 |
| | | 0.15 | 526. | 479. | 541. | 457. | 500.7 | 7.9 |
| | 4 | 0.00 | 25. | 29. | 25. | 30. | 27.2 | 9.7 |
| | | 0.05 | 155. | 163. | 132. | 106. | 139.0 | 18.4 |
| | | 0.10 | 329. | 226. | 256. | 278. | 272.2 | 15.9 |
| | | 0.15 | 457. | 399. | 477. | 439. | 443.0 | 7.5 |

TABLE 22

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 98

2026048970

502.00/ T501 /2059.00

A-/2059.00

| DATE | CON- DEN- SATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|-----------|-------------------------------|----------------|----------------------|------|------------|------|---------------------------|------------|
| | | | DIL.SUSP.3 | | DIL.SUSP.4 | | MEAN | RSD (%) |
| | | | PLATE 3.1 | 3.2 | 4.1 | 4.2 | | |
| | | (MG/ PLATE) | | | | | | |
| 10.NOV.81 | 54 | 0.00 | 108. | 118. | 105. | 123. | 113.5 | 7.4 |
| | | 0.05 | 153. | 153. | 166. | 139. | 152.7 | 7.2 |
| | | 0.10 | 176. | 219. | 192. | 192. | 194.7 | 9.2 |
| | | 0.15 | 224. | 218. | 236. | 221. | 224.7 | 3.5 |
| | 55 | 0.00 | 88. | 113. | 113. | 117. | 107.7 | 12.3 |
| | | 0.05 | 141. | 146. | 152. | 143. | 145.5 | 3.3 |
| | | 0.10 | 209. | 195. | 186. | 183. | 193.2 | 6.0 |
| | | 0.15 | 229. | 242. | 231. | 192. | 223.5 | 9.7 |
| 25.NOV.81 | 56 | 0.00 | 111. | 104. | 116. | 129. | 115.0 | 9.2 |
| | | 0.05 | 140. | 137. | 149. | 155. | 145.2 | 5.7 |
| | | 0.10 | 167. | 165. | 169. | 168. | 167.2 | 1.0 |
| | | 0.15 | 212. | 216. | 210. | 213. | 212.7 | 1.2 |
| | 57 | 0.00 | 110. | 108. | 117. | 118. | 113.2 | 4.4 |
| | | 0.05 | 154. | 148. | 168. | 146. | 154.0 | 6.5 |
| | | 0.10 | 175. | 174. | 207. | 177. | 183.2 | 8.7 |
| | | 0.15 | 195. | 210. | 202. | 216. | 205.7 | 4.5 |

TABLE 23

MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ T501 /2058.00

2026048971

A-/2059.00

| DATE | CON- DEN- SATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|-----------|-------------------------------|------|--------------------------|------|--------------|------|---------------------------|------------|
| | | | DIL. SUSP. 3 | | DIL. SUSP. 4 | | MEAN | RSD (%) |
| | | | PLATE (MG/ PLATED) | 3.1 | 3.2 | 4.1 | 4.2 | |
| 10.NOV.81 | 1 | 0.00 | 108. | 142. | 117. | 110. | 119.2 | 13.1 |
| | | 0.05 | 162. | 163. | 161. | 149. | 158.7 | 4.1 |
| | | 0.10 | 223. | 199. | 181. | 174. | 194.2 | 11.3 |
| | | 0.15 | 217. | 263. | 215. | 227. | 230.5 | 9.7 |
| | 2 | 0.00 | 105. | 120. | 127. | 104. | 114.0 | 10.0 |
| | | 0.05 | 149. | 158. | 205. | 151. | 165.7 | 16.0 |
| | | 0.10 | 209. | 227. | 214. | 183. | 208.2 | 8.9 |
| | | 0.15 | 261. | 243. | 224. | 220. | 237.0 | 8.0 |
| 25.NOV.81 | 3 | 0.00 | 110. | 112. | 125. | 116. | 115.7 | 5.7 |
| | | 0.05 | 178. | 163. | 181. | 152. | 168.5 | 8.0 |
| | | 0.10 | 199. | 206. | 218. | 228. | 212.7 | 6.0 |
| | | 0.15 | 262. | 243. | 238. | 254. | 248.2 | 4.3 |
| | 4 | 0.00 | 122. | 96. | 108. | 125. | 112.7 | 11.9 |
| | | 0.05 | 163. | 165. | 172. | 166. | 166.5 | 2.3 |
| | | 0.10 | 192. | 203. | 241. | 233. | 217.2 | 10.8 |
| | | 0.15 | 282. | 256. | 265. | 237. | 260.0 | 7.2 |

TABLE 24

MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION, STRAIN TA 100

2026048972

502.10/ T501 /2059.00

A-/2059.00

| DATE | CON- DEN- SATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|-----------|-------------------------------|----------------|----------------------|------|------------|------|---------------------------|------------|
| | | | DIL.SUSP.3 | | DIL.SUSP.4 | | MEAN | RSD (%) |
| | | | PLATE 3.1 | 3.2 | 4.1 | 4.2 | | |
| | | (MG/ PLATE) | | | | | | |
| 10.NOV.81 | 1 | 0.00 | 108. | 123. | 119. | 89. | 109.7 | 13.9 |
| | | 0.05 | 171. | 154. | 191. | 173. | 172.2 | 8.8 |
| | | 0.10 | 243. | 240. | 252. | 261. | 249.0 | 3.8 |
| | | 0.15 | 300. | 302. | 320. | 315. | 309.2 | 3.2 |
| | 2 | 0.00 | 109. | 116. | 113. | 115. | 113.2 | 2.7 |
| | | 0.05 | 178. | 167. | 215. | 169. | 182.2 | 12.3 |
| | | 0.10 | 207. | 248. | 223. | 230. | 252.0 | 15.1 |
| | | 0.15 | 362. | 324. | 324. | 336. | 336.5 | 5.3 |
| 25.NOV.81 | 3 | 0.00 | 110. | 129. | 125. | 125. | 124.7 | 8.5 |
| | | 0.05 | 169. | 185. | 217. | 166. | 184.2 | 12.7 |
| | | 0.10 | 279. | 266. | 265. | 226. | 259.0 | 8.8 |
| | | 0.15 | 340. | 323. | 321. | 336. | 330.0 | 2.9 |
| | 4 | 0.00 | 103. | 116. | 117. | 132. | 117.0 | 10.1 |
| | | 0.05 | 195. | 190. | 205. | 194. | 196.0 | 3.3 |
| | | 0.10 | 175. | 193. | 294. | 227. | 222.2 | 23.6 |
| | | 0.15 | 283. | 308. | 334. | 316. | 309.5 | 6.5 |

TABLE 25

MUTAGENICITY OF WSC-1 OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION, STRAIN TA 100

2026048973

502.10/ T501 /2059.00

A-2059.00

| DATE | CON- DEN- SATE BATCH | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL PARAMETERS | |
|-----------|-------------------------------|------|-------------------------|------|------------|------|---------------------------|------------|
| | | | DIL.SUSP.3 | | DIL.SUSP.4 | | MEAN | RSD (%) |
| | | | PLATE (MG/ PLATE) | 3.1 | 3.2 | 4.1 | 4.2 | |
| 10.NOV.81 | 1 | 0.00 | 123. | 103. | 131. | 126. | 120.7 | 10.2 |
| | | 0.05 | 177. | 161. | 172. | 162. | 168.0 | 4.6 |
| | | 0.10 | 184. | 196. | 231. | 252. | 215.7 | 14.5 |
| | | 0.15 | 250. | 250. | 274. | 237. | 252.7 | 6.1 |
| | 2 | 0.00 | 101. | 103. | 119. | 116. | 109.7 | 8.3 |
| | | 0.05 | 155. | 150. | 176. | 256. | 184.2 | 26.7 |
| | | 0.10 | 203. | 199. | 174. | 216. | 198.0 | 8.9 |
| | | 0.15 | 235. | 211. | 241. | 253. | 235.0 | 7.5 |
| 25.NOV.81 | 3 | 0.00 | 123. | - | 105. | 126. | 118.0 | 9.6 |
| | | 0.05 | 185. | 191. | 155. | 161. | 173.0 | 10.2 |
| | | 0.10 | 221. | 244. | 191. | 219. | 218.7 | 9.9 |
| | | 0.15 | 269. | 269. | 263. | 277. | 269.5 | 2.1 |
| | 4 | 0.00 | 120. | 116. | 114. | 94. | 111.0 | 10.5 |
| | | 0.05 | 166. | 187. | 158. | 164. | 168.7 | 7.5 |
| | | 0.10 | 200. | 221. | 174. | 192. | 196.7 | 9.9 |
| | | 0.15 | 259. | 243. | 260. | 261. | 255.7 | 3.3 |

TABLE 26

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 100

502.104 T501 A2059.00

2026048974

A-2059.00

| DATE | CON- | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL | | | |
|-----------|------|------|----------------------|-------|------------|------|-------------|-------|-----|------|
| | | | DIL.SUSP.3 | | DIL.SUSP.4 | | PARAMETERS | | | |
| | | | STATE | BATCH | PLATE | 3.1 | 3.2 | 4.1 | 4.2 | MEAN |
| | | | (MG/PLATE) | | | | | | | (%) |
| 10.NOV.81 | 1 | 0.00 | 126. | 102. | 106. | 118. | | 113.0 | | 9.7 |
| | | 0.05 | 185. | 146. | 145. | 135. | | 152.7 | | 14.4 |
| | | 0.10 | 271. | 230. | 220. | 192. | | 228.2 | | 14.3 |
| | | 0.15 | 287. | 281. | 277. | 272. | | 279.2 | | 2.3 |
| | 2 | 0.00 | 115. | 118. | 103. | 105. | | 110.2 | | 6.7 |
| | | 0.05 | 150. | 163. | 151. | 159. | | 155.7 | | 4.0 |
| | | 0.10 | 241. | 277. | 226. | 209. | | 238.2 | | 12.2 |
| | | 0.15 | 309. | 259. | 284. | 278. | | 282.5 | | 7.3 |
| 25.NOV.81 | 3 | 0.00 | 123. | 106. | 124. | 102. | | 113.7 | | 10.0 |
| | | 0.05 | 179. | 166. | 177. | 182. | | 176.0 | | 4.0 |
| | | 0.10 | 248. | 227. | 238. | 190. | | 225.7 | | 11.2 |
| | | 0.15 | 290. | 293. | 326. | 276. | | 296.2 | | 7.1 |
| | 4 | 0.00 | 120. | 120. | 112. | 93. | | 111.2 | | 11.4 |
| | | 0.05 | 189. | 175. | 181. | 189. | | 183.5 | | 3.7 |
| | | 0.10 | 249. | 238. | 260. | 187. | | 233.5 | | 13.8 |
| | | 0.15 | 303. | 305. | 292. | 278. | | 294.5 | | 6.2 |

TABLE 27

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 100

2026046975

502.10/ T501 /2059.00

A-2059.00

| DATE | CON- | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL | |
|-----------|------|------|----------------------|-------|------------|------------|-------------|------|
| | | | DEN- | | PARAMETERS | | | |
| | | | SATE | BATCH | DIL.SUSP.3 | DIL.SUSP.4 | PLATE | MEAN |
| | | | (MG/ | | | | | RSD |
| | | | PLATE | | | | (%) | |
| | | | 3.1 | 3.2 | 4.1 | 4.2 | | |
| 10.NOV.81 | 1 | 0.00 | 117. | 99. | 129. | 98. | 110.7 | 13.5 |
| | | 0.05 | 152. | 147. | 157. | 134. | 147.5 | 6.7 |
| | | 0.10 | 209. | 226. | 230. | 208. | 218.2 | 5.2 |
| | | 0.15 | 253. | 243. | 262. | 249. | 251.7 | 3.2 |
| | 2 | 0.00 | 103. | 104. | 122. | 115. | 110.2 | 9.1 |
| | | 0.05 | 149. | 123. | 127. | 129. | 132.0 | 8.8 |
| | | 0.10 | 208. | 185. | 205. | 188. | 196.5 | 5.9 |
| | | 0.15 | 251. | 219. | 254. | 215. | 234.7 | 8.8 |
| 25.NOV.81 | 3 | 0.00 | 105. | 121. | 90. | 112. | 107.0 | 12.2 |
| | | 0.05 | 157. | 159. | 182. | 155. | 163.2 | 7.7 |
| | | 0.10 | 215. | 193. | 189. | 194. | 197.7 | 5.9 |
| | | 0.15 | 226. | 220. | 234. | 219. | 224.7 | 3.1 |
| | 4 | 0.00 | 95. | 110. | 107. | 101. | 103.7 | 6.8 |
| | | 0.05 | 165. | 163. | 175. | 138. | 160.2 | 9.8 |
| | | 0.10 | 201. | 188. | 196. | 175. | 190.0 | 6.0 |
| | | 0.15 | 250. | 233. | 220. | 215. | 229.5 | 6.8 |

TABLE 28

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION, STRAIN TA 100

2026048976

502.10/ T501 /2059.00

A-/2059.00

| DATE | CON- | DOSE | REVERTANTS PER PLATE | | | | STATISTICAL | | | |
|-----------|------|------|----------------------|-------|------------|------------|-------------|------|------|-----|
| | | | DEN- | | PARAMETERS | | | | | |
| | | | SATE | BATCH | DIL.SUSP.3 | DIL.SUSP.4 | PLATE | MEAN | | |
| | | | (MG/PLATE) | | 3.1 | 3.2 | 4.1 | 4.2 | MEAN | RSD |
| | | | | | | | | | (%) | |
| 10.NOV.81 | 1 | 0.00 | 95. | 101. | 103. | 106. | 101.2 | 4.6 | | |
| | | 0.05 | 175. | 142. | 174. | 143. | 158.5 | 11.7 | | |
| | | 0.10 | 234. | 216. | 202. | 174. | 206.5 | 12.3 | | |
| | | 0.15 | 286. | 257. | 259. | 229. | 257.7 | 9.0 | | |
| | 2 | 0.00 | 105. | 123. | 111. | 128. | 116.7 | 9.1 | | |
| | | 0.05 | 151. | 158. | 159. | 147. | 153.7 | 3.7 | | |
| | | 0.10 | 206. | 223. | 205. | 189. | 205.7 | 6.8 | | |
| | | 0.15 | 279. | 252. | 280. | 279. | 272.5 | 5.0 | | |
| 25.NOV.81 | 3 | 0.00 | 107. | 115. | 103. | 115. | 110.0 | 5.5 | | |
| | | 0.05 | 143. | 164. | 136. | 152. | 148.7 | 8.1 | | |
| | | 0.10 | 207. | 193. | 194. | 213. | 201.0 | 5.3 | | |
| | | 0.15 | 224. | 268. | 209. | 259. | 240.0 | 11.7 | | |
| | 4 | 0.00 | 98. | 111. | 96. | 100. | 101.2 | 6.6 | | |
| | | 0.05 | 131. | 149. | 143. | 153. | 144.0 | 6.7 | | |
| | | 0.10 | 211. | 205. | 179. | 232. | 206.7 | 10.6 | | |
| | | 0.15 | 237. | 267. | 264. | 247. | 253.7 | 5.6 | | |

TABLE 29

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 100

2026048927

502.10/ T501 /2059.00

| CIGARETTE | DATE OF PLATE INCORPORATION MUTAGENICITY ASSAY | NUMBER OF COLONIES/ PLATE | NUMBER OF HISTIDINE PROTOTROPHS/ 10 COLONIES ASSAYED |
|-------------|---|---------------------------------|--|
| 2R1 | 9.Nov.81 24.Nov.81 | 283 258 | 10 10 |
| LEAR 0-17-2 | 9.Nov.81 24.Nov.81 | 329 407 | 10 10 |
| LEAR 0-17-3 | 9.Nov.81 24.Nov.81 | 479 556 | 10 10 |
| LEAR 2-17-2 | 9.Nov.81 24.Nov.81 | 254 364 | 10 10 |
| LEAR 2-17-3 | 9.Nov.81 24.Nov.81 | 433 487 | 10 10 |
| LEAR 4-17-2 | 9.Nov.81 24.Nov.81 | 280 362 | 10 10 |
| LEAR 4-17-3 | 9.Nov.81 24.Nov.81 | 506 479 | 10 10 |

TABLE 30

ASSAY FOR HISTIDINE PROTOTROPHY (REVERSION ASSAY) OF TA 98

colonies from plate incorporation mutagenicity assay plates

dates of determinations: 11.Nov.81
26.Nov.81

2026048978

| CIGARETTE | DATE OF PLATE INCORPORATION MUTAGENICITY ASSAY | NUMBER OF COLONIES/ PLATE | NUMBER OF HISTIDINE PROTOTROPHS/ 10 COLONIES ASSAYED |
|-------------|---|---------------------------------|--|
| 2R1 | 10.Nov.81 | 218 | 10 |
| | 25.Nov.81 | 216 | 10 |
| LEAR 0-17-2 | 10.Nov.81 | 263 | 10 |
| | 25.Nov.81 | 243 | 10 |
| LEAR 0-17-3 | 10.Nov.81 | 302 | 10 |
| | 25.Nov.81 | 323 | 10 |
| LEAR 2-17-2 | 10.Nov.81 | 250 | 10 |
| | 25.Nov.81 | 269 | 10 |
| LEAR 2-17-3 | 10.Nov.81 | 281 | 10 |
| | 25.Nov.81 | 293 | 10 |
| LEAR 4-17-2 | 10.Nov.81 | 243 | 10 |
| | 25.Nov.81 | 220 | 10 |
| LEAR 4-17-3 | 10.Nov.81 | 257 | 10 |
| | 25.Nov.81 | 268 | 10 |

TABLE 31

ASSAY FOR HISTIDINE PROTOTROPHY (REVERSION ASSAY) OF TA 100

colonies from plate incorporation mutagenicity assay plates

dates of determinations: 12.Nov.81
27.Nov.81

2026048979

A-/2059.00

| DATE | DOSE | REVERTANTS PER PLATE | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|----------------|------------------------|-------|------|-----------------|-------------------|
| | | STATISTICAL PARAMETERS | N | MEAN | RSO (%) | |
| | (MG/ PLATE) | | | | | |
| 9.NOV.81 | 0.00 | 8 | 32.0 | 23.5 | | |
| | 0.05 | 8 | 100.3 | 5.7 | | |
| | 0.10 | 8 | 193.2 | 6.7 | | |
| | 0.15 | 8 | 257.3 | 7.0 | 1538. | 0.989 |
| 24.NOV.81 | 0.00 | 8 | 28.7 | 9.8 | | |
| | 0.05 | 8 | 99.2 | 15.9 | | |
| | 0.10 | 8 | 177.5 | 6.3 | | |
| | 0.15 | 8 | 258.2 | 2.8 | 1533. | 0.993 |
| 9.NOV.81 | 0.00 | 16 | 30.4 | 18.9 | | |
| 24.NOV.81 | 0.05 | 16 | 99.8 | 11.5 | | |
| | 0.10 | 16 | 185.4 | 7.7 | | |
| | 0.15 | 16 | 257.8 | 5.1 | 1536. | 0.991 |

TABLE 32

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ F501 /2058.00

2026048980

A-/2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE STATISTICAL PARAMETERS | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|------------------------|--|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 9.NOV.81 | 0.00 | 8 | 31.3 | 35.7 | 1966. | 0.991 |
| | 0.05 | 8 | 105.8 | 14.1 | | |
| | 0.10 | 8 | 212.1 | 3.6 | | |
| | 0.15 | 8 | 323.6 | 4.1 | | |
| 24.NOV.81 | 0.00 | 8 | 32.7 | 22.9 | 2505. | 0.985 |
| | 0.05 | 8 | 119.5 | 10.4 | | |
| | 0.10 | 8 | 269.0 | 10.5 | | |
| | 0.15 | 8 | 400.3 | 6.9 | | |
| 9.NOV.81 | 0.00 | 16 | 32.1 | 28.8 | 2235. | 0.970 |
| | 0.05 | 16 | 112.7 | 13.3 | | |
| | 0.10 | 16 | 240.6 | 14.8 | | |
| | 0.15 | 16 | 362.0 | 12.4 | | |

TABLE 33

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ F501 /2059.00

2026048981

A-2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE STATISTICAL PARAMETERS | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|------------------------|--|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 9.NOV.81 | 0.00 | 8 | 29.2 | 24.5 | 3354. | 0.976 |
| | 0.05 | 8 | 144.7 | 18.3 | | |
| | 0.10 | 8 | 355.3 | 10.5 | | |
| | 0.15 | 8 | 518.0 | 12.3 | | |
| 24.NOV.81 | 0.00 | 8 | 28.7 | 23.9 | 3662. | 0.979 |
| | 0.05 | 8 | 180.7 | 8.2 | | |
| | 0.10 | 8 | 405.5 | 4.4 | | |
| | 0.15 | 8 | 564.2 | 14.5 | | |
| 9.NOV.81 | 0.00 | 16 | 29.0 | 23.4 | 3508. | 0.973 |
| | 0.05 | 16 | 162.8 | 17.1 | | |
| | 0.10 | 16 | 380.4 | 10.1 | | |
| | 0.15 | 16 | 541.1 | 13.8 | | |

TABLE 34

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ F501 /2059.00

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A-/2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE STATISTICAL PARAMETERS | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|------------------------|--|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 9.NOV.81 | 0.00 | 8 | 32.1 | 16.2 | 1760. | 0.979 |
| | 0.05 | 8 | 96.1 | 20.1 | | |
| | 0.10 | 8 | 187.3 | 9.3 | | |
| | 0.15 | 8 | 295.1 | 8.6 | | |
| 24.NOV.81 | 0.00 | 8 | 27.3 | 18.9 | 2099. | 0.977 |
| | 0.05 | 8 | 109.7 | 16.3 | | |
| | 0.10 | 8 | 216.8 | 15.8 | | |
| | 0.15 | 8 | 341.5 | 8.8 | | |
| 9.NOV.81 | 0.00 | 16 | 29.8 | 18.8 | 1930. | 0.970 |
| | 0.05 | 16 | 102.9 | 18.8 | | |
| | 0.10 | 16 | 202.1 | 15.0 | | |
| | 0.15 | 16 | 318.3 | 11.3 | | |

TABLE 35

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ F501 /2059.00

2026048983

A-/2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE STATISTICAL PARAMETERS: | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|------------------------|---|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 9.NOV.81 | 0.00 | 8 | 25.8 | 18.4 | 2869. | 0.976 |
| | 0.05 | 8 | 146.0 | 11.0 | | |
| | 0.10 | 8 | 311.3 | 15.1 | | |
| | 0.15 | 8 | 448.8 | 12.1 | | |
| 24.NOV.81 | 0.00 | 8 | 31.2 | 19.7 | 3016. | 0.990 |
| | 0.05 | 8 | 162.6 | 7.6 | | |
| | 0.10 | 8 | 336.5 | 9.0 | | |
| | 0.15 | 8 | 476.0 | 7.3 | | |
| 9.NOV.81 | 0.00 | 16 | 28.6 | 21.0 | 2942. | 0.981 |
| | 0.05 | 16 | 154.3 | 10.6 | | |
| | 0.10 | 16 | 323.9 | 12.4 | | |
| | 0.15 | 16 | 462.4 | 10.0 | | |

TABLE 36

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ F501 /2059.00

2026048984

A-/2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE STATISTICAL PARAMETERS | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|------------------------|--|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 9.NOV.81 | 0.00 | 8 | 28.1 | 28.2 | 2019. | 0.982 |
| | 0.05 | 8 | 101.2 | 8.1 | | |
| | 0.10 | 8 | 227.6 | 7.5 | | |
| | 0.15 | 8 | 322.5 | 10.6 | | |
| 24.NOV.81 | 0.00 | 8 | 31.1 | 13.4 | 2026. | 0.968 |
| | 0.05 | 8 | 116.3 | 8.2 | | |
| | 0.10 | 8 | 226.1 | 17.0 | | |
| | 0.15 | 8 | 332.2 | 14.0 | | |
| 9.NOV.81 | 0.00 | 16 | 29.6 | 24.3 | 2023. | 0.975 |
| | 0.05 | 16 | 108.8 | 10.7 | | |
| | 0.10 | 16 | 226.9 | 12.7 | | |
| | 0.15 | 16 | 327.4 | 12.2 | | |

TABLE 37

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ F501 /2059.00

2026048985

A-/2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE STATISTICAL PARAMETERS | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|------------------------|--|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 9.NOV.81 | 0.00 | 8 | 29.2 | 14.7 | 3014. | 0.984 |
| | 0.05 | 6 | 117.1 | 25.2 | | |
| | 0.10 | 8 | 308.3 | 6.7 | | |
| | 0.15 | 8 | 471.2 | 6.8 | | |
| 24.NOV.81 | 0.00 | 8 | 28.7 | 15.7 | 2963. | 0.978 |
| | 0.05 | 8 | 142.6 | 17.3 | | |
| | 0.10 | 8 | 294.6 | 14.4 | | |
| | 0.15 | 8 | 471.8 | 9.7 | | |
| 9.NOV.81 | 0.00 | 16 | 29.0 | 14.7 | 2988. | 0.981 |
| | 0.05 | 14 | 131.7 | 22.3 | | |
| | 0.10 | 16 | 301.5 | 10.9 | | |
| | 0.15 | 16 | 471.6 | 8.1 | | |

TABLE 38

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 98

502.00/ F501 /2059.00

2026048986

A-/2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE STATISTICAL PARAMETERS | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|------------------------|--|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 10.NOV.81 | 0.00 | 8 | 110.6 | 9.7 | 771. | 0.963 |
| | 0.05 | 8 | 149.1 | 5.9 | | |
| | 0.10 | 8 | 194.0 | 7.2 | | |
| | 0.15 | 8 | 224.1 | 6.8 | | |
| 25.NOV.81 | 0.00 | 8 | 114.1 | 6.7 | 622. | 0.965 |
| | 0.05 | 8 | 149.6 | 6.5 | | |
| | 0.10 | 8 | 175.2 | 7.7 | | |
| | 0.15 | 8 | 209.2 | 3.5 | | |
| 10.NOV.81 | 0.00 | 16 | 112.4 | 8.2 | 696. | 0.954 |
| | 0.05 | 16 | 149.4 | 6.0 | | |
| 25.NOV.81 | 0.10 | 16 | 184.6 | 8.9 | | |
| | 0.15 | 16 | 216.7 | 6.4 | | |

TABLE 39

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2058.00

2026048987

A-/2059.00

| DATE | DOSE | REVERTANTS PER PLATE | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|----------------|----------------------|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| | (MG/ PLATE) | | | | | |
| 10.NOV.81 | 0.00 | 8 | 116.6 | 11.1 | | |
| | 0.05 | 8 | 162.2 | 11.2 | | |
| | 0.10 | 8 | 201.2 | 10.0 | | |
| | 0.15 | 8 | 233.7 | 8.3 | 781. | 0.931 |
| 25.NOV.81 | 0.00 | 8 | 114.2 | 8.7 | | |
| | 0.05 | 8 | 167.5 | 5.5 | | |
| | 0.10 | 8 | 215.0 | 8.2 | | |
| | 0.15 | 8 | 254.6 | 6.0 | 937. | 0.970 |
| 10.NOV.81 | 0.00 | 16 | 115.4 | 9.7 | | |
| 25.NOV.81 | 0.05 | 16 | 164.9 | 8.6 | | |
| | 0.10 | 16 | 208.1 | 9.4 | | |
| | 0.15 | 16 | 244.2 | 8.2 | 839. | 0.946 |

TABLE 4o

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048988

A-/2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE STATISTICAL PARAMETERS | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|------------------------|--|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 10.NOV.81 | 0.00 | 8 | 111.5 | 9.3 | 1415. | 0.974 |
| | 0.05 | 8 | 177.2 | 10.4 | | |
| | 0.10 | 8 | 250.5 | 10.3 | | |
| | 0.15 | 8 | 322.8 | 6.1 | | |
| 25.NOV.81 | 0.00 | 8 | 120.8 | 9.3 | 1294. | 0.948 |
| | 0.05 | 8 | 190.1 | 9.0 | | |
| | 0.10 | 8 | 240.6 | 17.6 | | |
| | 0.15 | 8 | 319.7 | 5.7 | | |
| 10.NOV.81 | 0.00 | 16 | 116.2 | 9.9 | 1354. | 0.961 |
| | 0.05 | 16 | 183.7 | 10.0 | | |
| | 0.10 | 16 | 245.6 | 13.9 | | |
| | 0.15 | 16 | 321.3 | 5.7 | | |

TABLE 41

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F504 /2059.00

2026048989

A-/2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE | | | REGR. COEFF. | CORREL. COEFF. |
|------------------------|------------------------|----------------------|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 10.NOV.81 | 0.00 | 8 | 115.2 | 10.1 | 833. | 0.894 |
| | 0.05 | 8 | 176.1 | 19.1 | | |
| | 0.10 | 8 | 206.8 | 12.3 | | |
| | 0.15 | 8 | 243.8 | 7.4 | | |
| 25.NOV.81 | 0.00 | 7 | 114.0 | 9.8 | 964. | 0.962 |
| | 0.05 | 8 | 170.8 | 8.4 | | |
| | 0.10 | 8 | 207.7 | 10.8 | | |
| | 0.15 | 8 | 262.6 | 3.8 | | |
| 10.NOV.81 25.NOV.81 | 0.00 | 15 | 114.7 | 9.6 | 897. | 0.928 |
| | 0.05 | 16 | 173.5 | 14.5 | | |
| | 0.10 | 16 | 207.3 | 11.2 | | |
| | 0.15 | 16 | 253.3 | 6.7 | | |

TABLE 42

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048390

A-72059.00

| DATE | DOSE | REVERTANTS PER PLATE | | | REGR. | CORREL. |
|-----------|------------|------------------------|-------|--------|-------|---------|
| | | STATISTICAL PARAMETERS | MEAN | RSD | | |
| | (MG/PLATE) | N | (%) | (1/MG) | | |
| 10.NOV.81 | 0.00 | 8 | 111.6 | 7.9 | | |
| | 0.05 | 8 | 154.2 | 9.8 | | |
| | 0.10 | 8 | 233.2 | 12.5 | | |
| | 0.15 | 8 | 280.8 | 5.1 | 1173. | 0.961 |
| 25.NOV.81 | 0.00 | 8 | 112.5 | 10.0 | | |
| | 0.05 | 8 | 179.7 | 4.2 | | |
| | 0.10 | 8 | 229.6 | 11.8 | | |
| | 0.15 | 8 | 295.3 | 5.4 | 1197. | 0.971 |
| 10.NOV.81 | 0.00 | 16 | 112.1 | 8.7 | | |
| 25.NOV.81 | 0.05 | 16 | 167.0 | 10.5 | | |
| | 0.10 | 16 | 231.4 | 11.8 | | |
| | 0.15 | 16 | 288.1 | 5.7 | 1185. | 0.964 |

TABLE 43

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048991

A-/2059.00

| DATE | DOSE | REVERTANTS PER PLATE | | | REGR. | CORREL. |
|-----------|------------|----------------------|-------|---------|--------|---------|
| | | N | MEAN | RSD (%) | | |
| | (MG/PLATE) | | | | COEFF. | |
| 10.NOV.81 | 0.00 | 8 | 110.5 | 10.7 | | |
| | 0.05 | 8 | 139.7 | 9.3 | | |
| | 0.10 | 8 | 207.3 | 7.6 | | |
| | 0.15 | 8 | 243.2 | 7.0 | 932. | 0.957 |
| 25.NOV.81 | 0.00 | 8 | 105.3 | 9.4 | | |
| | 0.05 | 8 | 161.7 | 8.2 | | |
| | 0.10 | 8 | 193.8 | 5.9 | | |
| | 0.15 | 8 | 227.1 | 5.0 | 795. | 0.962 |
| 10.NOV.81 | 0.00 | 16 | 107.9 | 10.0 | | |
| 25.NOV.81 | 0.05 | 16 | 150.8 | 11.3 | | |
| | 0.10 | 16 | 200.6 | 7.5 | | |
| | 0.15 | 16 | 235.2 | 6.9 | 863. | 0.956 |

TABLE 44

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION, STRAIN TA 100

502.104 F501 /2059.00

2026048992

A-/2059.00

| DATE | DOSE | REVERTANTS PER PLATE | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|----------------|----------------------|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSO (%) | | |
| | (MG/ PLATE) | | | | | |
| 10.NOV.81 | 0.00 | 8 | 109.0 | 10.3 | | |
| | 0.05 | 8 | 156.1 | 8.3 | | |
| | 0.10 | 8 | 206.1 | 9.2 | | |
| | 0.15 | 8 | 265.1 | 7.3 | 1037. | 0.967 |
| 25.NOV.81 | 0.00 | 8 | 105.6 | 7.1 | | |
| | 0.05 | 8 | 146.3 | 7.1 | | |
| | 0.10 | 8 | 203.8 | 7.9 | | |
| | 0.15 | 8 | 246.8 | 8.9 | 962. | 0.965 |
| 10.NOV.81 | 0.00 | 16 | 107.3 | 8.8 | | |
| 25.NOV.81 | 0.05 | 16 | 151.3 | 8.2 | | |
| | 0.10 | 16 | 205.0 | 8.3 | | |
| | 0.15 | 16 | 256.0 | 8.6 | 1000. | 0.963 |

TABLE 45

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048993

A-2059.00

| DATE | DOSE | REVERTANTS PER PLATE | | | REGR. | CORREL. |
|-----------|------------|------------------------|-------|--------|-------|---------|
| | | STATISTICAL PARAMETERS | MEAN | RSD | | |
| | (MG/PLATE) | N | (%) | (1/MG) | | |
| 10.NOV.81 | 0.00 | 8 | 116.6 | 11.1 | | |
| | 0.05 | 8 | 162.2 | 11.2 | | |
| | 0.10 | 8 | 201.2 | 10.0 | | |
| | 0.15 | 8 | 233.7 | 8.3 | 781. | 0.931 |
| 25.NOV.81 | 0.00 | 8 | 114.2 | 8.7 | | |
| | 0.05 | 8 | 167.5 | 5.5 | | |
| | 0.10 | 8 | 215.0 | 8.2 | | |
| | 0.15 | 8 | 254.6 | 6.0 | 937. | 0.970 |
| 10.NOV.81 | 0.00 | 16 | 115.4 | 9.7 | | |
| 25.NOV.81 | 0.05 | 16 | 164.9 | 8.6 | | |
| | 0.10 | 16 | 208.1 | 9.4 | | |
| | 0.15 | 16 | 244.2 | 8.2 | 859. | 0.946 |

TABLE 4o

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION, STRAIN TA 100

502.107 F501 /2059.00

2026048994

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KOELN

A-/2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE STATISTICAL PARAMETERS | | | REGR. COEFF. (1/MG) | CORREL. COEFF. |
|-----------|------------------------|--|-------|------------|---------------------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 10.NOV.81 | 0.00 | 8 | 111.5 | 9.3 | 1415. | 0.974 |
| | 0.05 | 8 | 177.2 | 10.4 | | |
| | 0.10 | 8 | 250.5 | 10.3 | | |
| | 0.15 | 8 | 322.8 | 6.1 | | |
| 25.NOV.81 | 0.00 | 8 | 120.8 | 9.3 | 1294. | 0.948 |
| | 0.05 | 8 | 190.1 | 9.0 | | |
| | 0.10 | 8 | 240.6 | 17.6 | | |
| | 0.15 | 8 | 319.7 | 5.7 | | |
| 10.NOV.81 | 0.00 | 16 | 116.2 | 9.9 | | |
| 25.NOV.81 | 0.05 | 16 | 183.7 | 10.0 | | |
| | 0.10 | 16 | 245.6 | 13.9 | | |
| | 0.15 | 16 | 321.3 | 5.7 | 1354. | 0.961 |

TABLE 41

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048995

A-72059.00

| DATE | DOSE | REVERTANTS PER PLATE | | | REGR. | CORREL. |
|-----------|------------|------------------------|-------|------|---------|---------|
| | | STATISTICAL PARAMETERS | N | MEAN | RSD (%) | COEFF. |
| | (MG/PLATE) | | | | | |
| 10.NOV.81 | 0.00 | 8 | 115.2 | 10.1 | | |
| | 0.05 | 8 | 176.1 | 19.1 | | |
| | 0.10 | 8 | 206.8 | 12.3 | | |
| | 0.15 | 8 | 243.8 | 7.4 | 833. | 0.394 |
| 25.NOV.81 | 0.00 | 7 | 114.0 | 9.8 | | |
| | 0.05 | 8 | 170.8 | 8.4 | | |
| | 0.10 | 8 | 207.7 | 10.8 | | |
| | 0.15 | 8 | 262.6 | 3.8 | 964. | 0.962 |
| 10.NOV.81 | 0.00 | 15 | 114.7 | 9.6 | | |
| 25.NOV.81 | 0.05 | 16 | 173.5 | 14.5 | | |
| | 0.10 | 16 | 207.3 | 11.2 | | |
| | 0.15 | 16 | 253.3 | 6.7 | 897. | 0.928 |

TABLE 42

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048996

A-2059.00

| DATE | DOSE | REVERTANTS PER PLATE | | | REGR. | CORREL. |
|-----------|------------|----------------------|-------|------|-------|---------|
| | | N | MEAN | RSD | | |
| | (MG/PLATE) | | | (%) | | (1/MG) |
| 10.NOV.81 | 0.00 | 8 | 111.6 | 7.9 | 1173. | 0.964 |
| | 0.05 | 8 | 154.2 | 9.8 | | |
| | 0.10 | 8 | 233.2 | 12.5 | | |
| | 0.15 | 8 | 280.8 | 5.1 | | |
| 25.NOV.81 | 0.00 | 8 | 142.5 | 10.0 | 1197. | 0.971 |
| | 0.05 | 8 | 179.7 | 4.2 | | |
| | 0.10 | 8 | 229.6 | 11.8 | | |
| | 0.15 | 8 | 295.3 | 5.4 | | |
| 10.NOV.81 | 0.00 | 16 | 112.1 | 8.7 | 1185. | 0.964 |
| | 0.05 | 16 | 167.0 | 10.5 | | |
| | 0.10 | 16 | 231.4 | 11.8 | | |
| | 0.15 | 16 | 288.1 | 5.7 | | |

TABLE 43:

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026046997

A-12059.00

| DATE | DOSE | REVERTANTS PER PLATE | | | REGR. | CORREL. |
|-----------|------------|----------------------|-------|---------|--------|---------|
| | | N | MEAN | RSD (%) | | |
| | (MG/PLATE) | | | | COEFF. | (1/MG) |
| 10.NOV.81 | 0.00 | 8 | 110.5 | 10.7 | 932. | 0.957 |
| | 0.05 | 8 | 139.7 | 9.3 | | |
| | 0.10 | 8 | 207.3 | 7.6 | | |
| | 0.15 | 8 | 243.2 | 7.0 | | |
| 25.NOV.81 | 0.00 | 8 | 105.3 | 9.4 | 795. | 0.962 |
| | 0.05 | 8 | 161.7 | 8.2 | | |
| | 0.10 | 8 | 193.8 | 5.9 | | |
| | 0.15 | 8 | 227.1 | 5.0 | | |
| 10.NOV.81 | 0.00 | 16 | 107.9 | 10.0 | 863. | 0.958 |
| | 0.05 | 16 | 150.8 | 11.3 | | |
| | 0.10 | 16 | 200.6 | 7.5 | | |
| | 0.15 | 16 | 235.2 | 6.9 | | |

TABLE 44

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION, STRAIN TA 100

502.101 F501 /2059.00

2026048998

A-2059.00

| DATE | DOSE (MG/ PLATE) | REVERTANTS PER PLATE | | | REGR. COEFF. | CORREL. COEFF. |
|-----------|------------------------|----------------------|-------|------------|-----------------|-------------------|
| | | N | MEAN | RSD (%) | | |
| 10.NOV.81 | 0.00 | 8 | 109.0 | 10.3 | 1037. | 0.967 |
| | 0.05 | 8 | 156.1 | 8.3 | | |
| | 0.10 | 8 | 206.1 | 9.2 | | |
| | 0.15 | 8 | 265.1 | 7.3 | | |
| 25.NOV.81 | 0.00 | 8 | 105.6 | 7.1 | 962. | 0.965 |
| | 0.05 | 8 | 146.3 | 7.1 | | |
| | 0.10 | 8 | 203.8 | 7.9 | | |
| | 0.15 | 8 | 246.8 | 8.9 | | |
| 10.NOV.81 | 0.00 | 16 | 107.3 | 8.8 | 1000. | 0.963 |
| | 0.05 | 16 | 151.3 | 8.2 | | |
| 25.NOV.81 | 0.10 | 16 | 205.0 | 8.3 | | |
| | 0.15 | 16 | 256.0 | 8.6 | | |

TABLE 45

SPECIFIC MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 100

502.10/ F501 /2059.00

2026048999

| A CIGARETTE | B SPECIFIC MUTAGENICITY (rev./mg) | C | D | E | | | | |
|----------------|---|------|------|------|---|------|--------------------------------------|-------------------------------------|
| | | | | 1 | 2 | MEAN | RELATIVE DIFFERENCE $((B + C)/2)$ | ABSOLUTE $((B - C)/D)$.GE. 0.25 |
| ASSAY | | | | | | | | |
| 2R1 | 1538 | 1533 | 1536 | 0.00 | 0 | | | |
| LEAR 0-17-2 | 1966 | 2505 | 2236 | 0.24 | 0 | | | |
| LEAR 0-17-3 | 3354 | 3662 | 3508 | 0.09 | 0 | | | |
| LEAR 2-17-2 | 1760 | 2099 | 1930 | 0.18 | 0 | | | |
| LEAR 2-17-3 | 2869 | 3016 | 2943 | 0.05 | 0 | | | |
| LEAR 4-17-2 | 2019 | 2026 | 2023 | 0.00 | 0 | | | |
| LEAR 4-17-3 | 3014 | 2963 | 2989 | 0.02 | 0 | | | |

TABLE 46

STATISTICAL SIGNIFICANCE BETWEEN 2 INDEPENDENT ASSAYS,
STRAIN TA 98

2026043000

| A | B | C | D | E |
|-------------|------------------------------------|------|---------------|---|
| CIGARETTE | SPECIFIC MUTAGENICITY (rev./mg) | | | |
| ASSAY | | | | |
| | 1 | 2 | MEAN | RELATIVE DIFFERENCE |
| | | | $((B + C)/2)$ | $((B - C)/D) \cdot GE.0.25$ ABSOLUTE |
| 2R1 | 771 | 622 | 697 | 0.21 |
| LEAR 0-17-2 | 781 | 937 | 859 | 0.18 |
| LEAR 0-17-3 | 1415 | 1294 | 1355 | 0.09 |
| LEAR 2-17-2 | 833 | 964 | 899 | 0.15 |
| LEAR 2-17-3 | 1173 | 1197 | 1185 | 0.02 |
| LEAR 4-17-2 | 932 | 795 | 864 | 0.16 |
| LEAR 4-17-3 | 1037 | 962 | 1000 | 0.08 |

TABLE 47

STATISTICAL SIGNIFICANCE BETWEEN 2 INDEPENDENT ASSAYS,
STRAIN TA 100

2026049001

| CIGARETTE | SPECIFIC MUTAGENICITY (rev./mg) | CORREL. COEFF. | RELATIVE SPEC. MUTAGENICITY (%) |
|-------------|---------------------------------------|-------------------|---------------------------------------|
| 2R1 | 1536 | 0.991 | 100.0 (a) |
| LEAR 0-17-2 | 2235 | 0.970 | 145.5 |
| LEAR 0-17-3 | 3508 | 0.973 | 228.4 |
| LEAR 2-17-2 | 1930 | 0.970 | 125.7 |
| LEAR 2-17-3 | 2942 | 0.981 | 191.5 |
| LEAR 4-17-2 | 2023 | 0.975 | 131.7 |
| LEAR 4-17-3 | 2988 | 0.981 | 194.5 |

TABLE 48

SPECIFIC AND RELATIVE SPECIFIC MUTAGENICITY OF WSC-I,
STRAIN TA 98

2026049002

(a) specific mutagenicity of WSC-I of 2R1 set to 100 %

| CIGARETTE | SPECIFIC MUTAGENICITY (rev./mg) | CORREL. COEFF. | RELATIVE SPEC. MUTAGENICITY (%) |
|-------------|---------------------------------------|-------------------|---------------------------------------|
| 2R1 | 696 | 0.954 | 100.0 (a) |
| LEAR 0-17-2 | 859 | 0.946 | 123.4 |
| LEAR 0-17-3 | 1354 | 0.961 | 194.5 |
| LEAR 2-17-2 | 897 | 0.928 | 128.9 |
| LEAR 2-17-3 | 1185 | 0.964 | 170.3 |
| LEAR 4-17-2 | 863 | 0.956 | 124.0 |
| LEAR 4-17-3 | 1000 | 0.963 | 143.7 |

TABLE 49

SPECIFIC AND RELATIVE SPECIFIC MUTAGENICITY OF WSC-I,
STRAIN TA 100

2026049003

(a) specific mutagenicity of WSC-I of 2R1 set to 100 %

| CIGARETTE TYPE | 2R1 | LEAR o-17-2 | LEAR o-17-3 | LEAR 2-17-2 | LEAR 2-17-3 | LEAR 4-17-2 | LEAR 4-17-3 |
|-------------------|-----|----------------|----------------|----------------|----------------|----------------|----------------|
| 2R1 | - | 1 | 1 | 1 | 1 | 1 | 1 |
| LEAR o-17-2 | - | - | 1 | o | 1 | o | 1 |
| LEAR o-17-3 | - | - | - | 2 | 2 | 2 | 2 |
| LEAR 2-17-2 | - | - | - | - | 1 | o | 1 |
| LEAR 2-17-3 | - | - | - | - | - | 2 | o |
| LEAR 4-17-2 | - | - | - | - | - | - | 1 |
| LEAR 4-17-3 | - | - | - | - | - | - | - |

TABLE 5o

STATISTICAL SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE
SPECIFIC MUTAGENICITIES OF 2 INDIVIDUAL CIGARETTES,
STRAIN TA 98

- o : no statistically significant difference
- 1 : mutagenicity of cigarette given in headline
higher than mutagenicity of cigarette given
in column
- 2 : mutagenicity of cigarette given in headline
lower than mutagenicity of cigarette given
in column

2026043004

| CIGARETTE TYPE | 2R1 | LEAR o-17-2 | LEAR o-17-3 | LEAR 2-17-2 | LEAR 2-17-3 | LEAR 4-17-2 | LEAR 4-17-3 |
|-------------------|-----|----------------|----------------|----------------|----------------|----------------|----------------|
| 2R1 | - | 1 | 1 | 1 | 1 | 1 | 1 |
| LEAR o-17-2 | - | 1 | o | 1 | o | o | o |
| LEAR o-17-3 | | - | 2 | o | 2 | 2 | |
| LEAR 2-17-2 | | | - | 1 | o | o | |
| LEAR 2-17-3 | | | | - | 2 | 2 | |
| LEAR 4-17-2 | | | | | - | o | |
| LEAR 4-17-3 | | | | | | - | |

TABLE 51

STATISTICAL SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE
SPECIFIC MUTAGENICITIES OF 2 INDIVIDUAL CIGARETTES,
STRAIN TA 100

- o : no statistically significant difference
- 1 : mutagenicity of cigarette given in headline
higher than mutagenicity of cigarette given
in column
- 2 : mutagenicity of cigarette given in headline
lower than mutagenicity of cigarette given
in column

2026043005

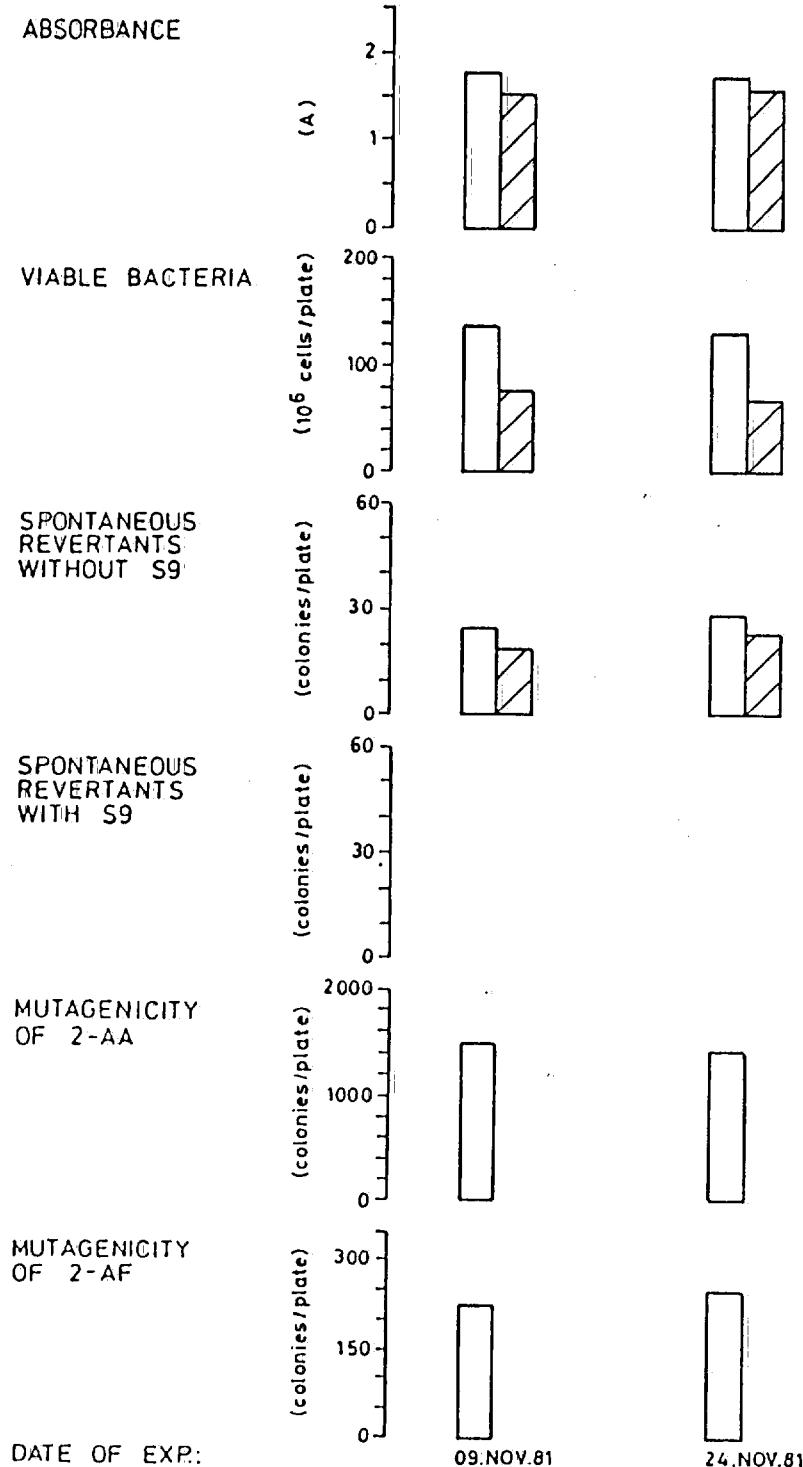


FIGURE 1

PROPERTIES OF TA 98 SUSPENSION CULTURES, test performed at the beginning (□) and at the end (▨) of each mutagenicity assay (see TABLES 5, 6 and 9)

2026049006

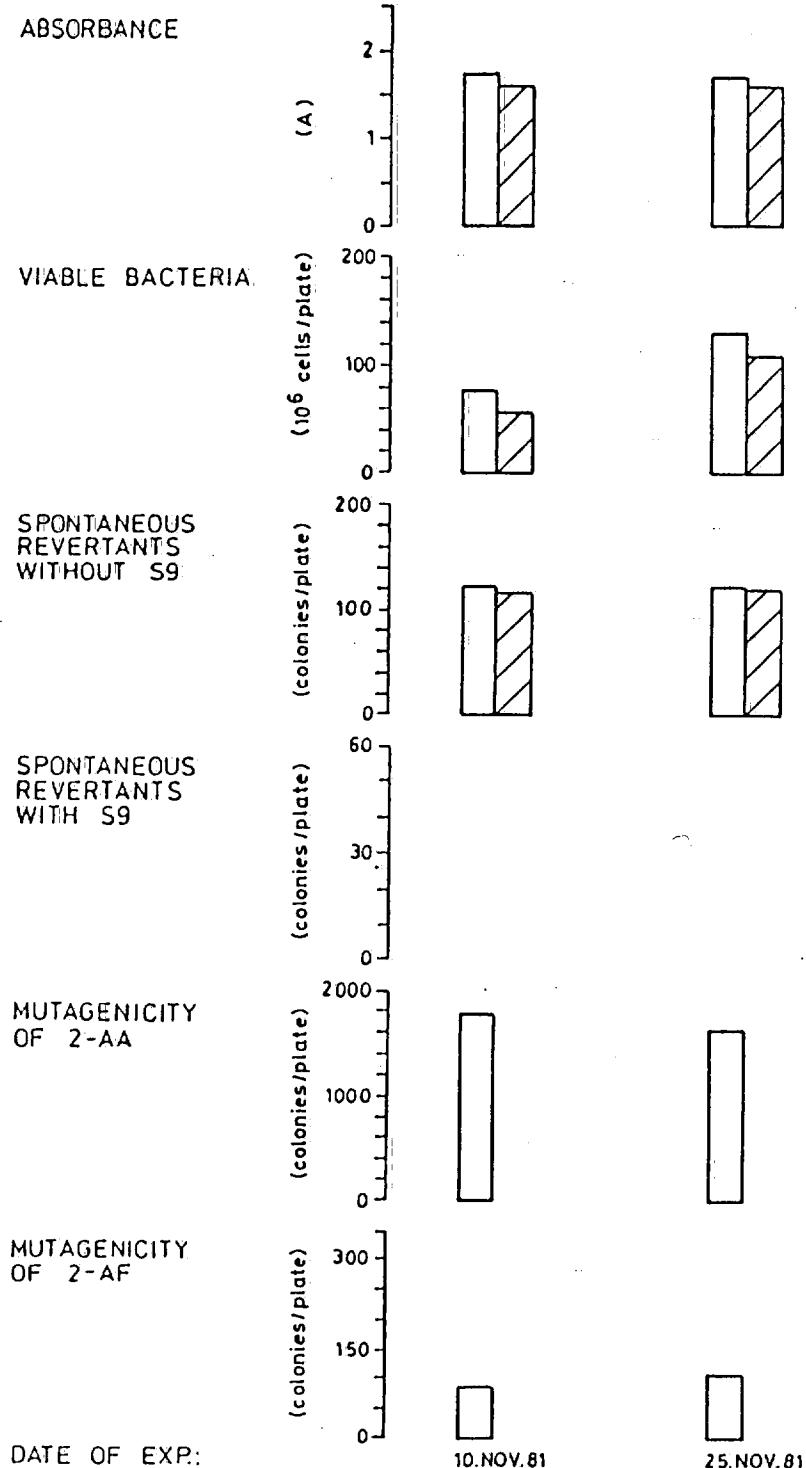


FIGURE 2

PROPERTIES OF TA 100 SUSPENSION CULTURES, test performed at the beginning (□) and at the end (▨) of each mutagenicity assay (see TABLES 7, 8 and 10)

20268049007

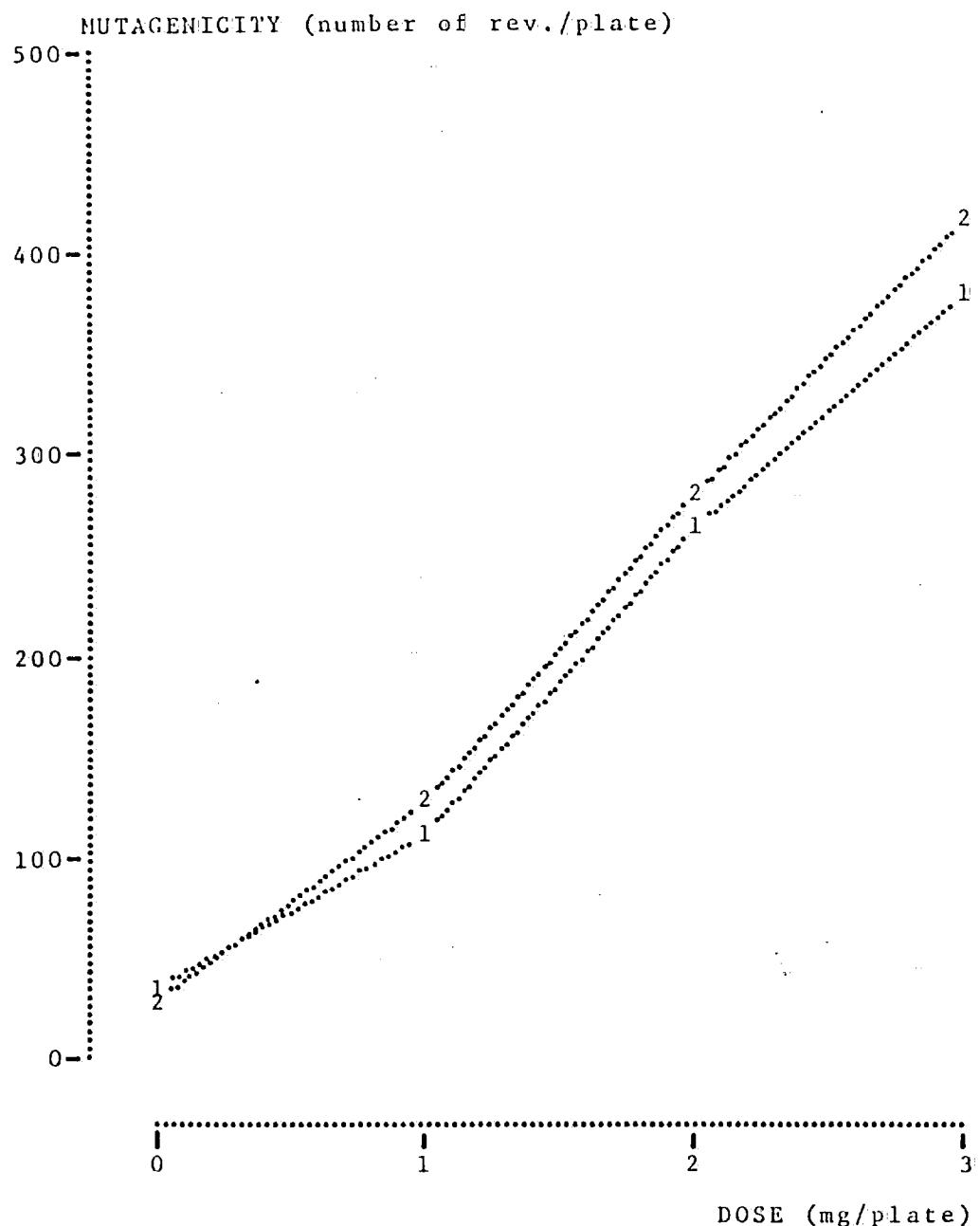


FIGURE 3

MUTAGENICITY OF 2-AMINOFLUORENE WITH S9 ACTIVATION, STRAIN TA 98
(see TABLE 11)

2026043008

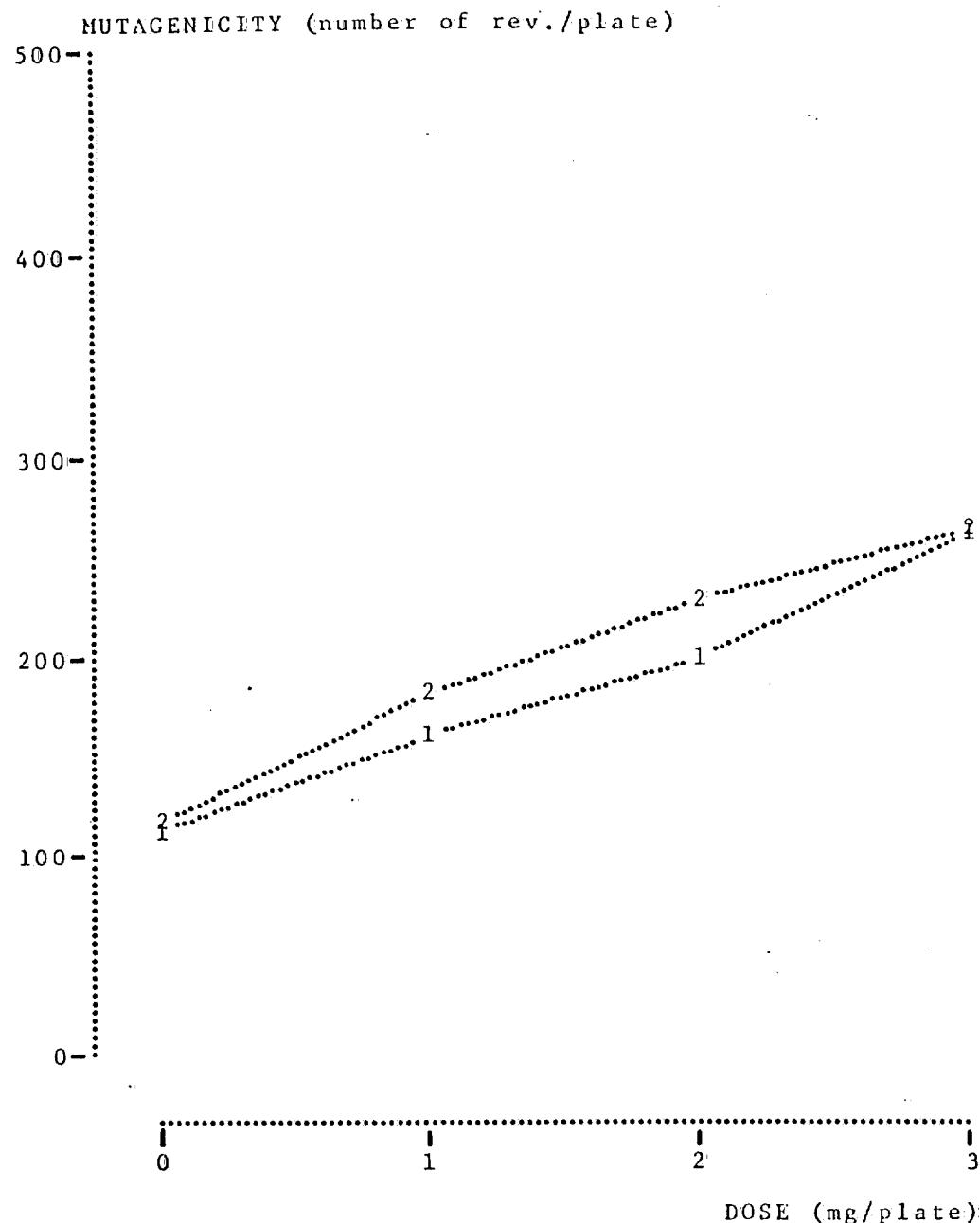


FIGURE 4

MUTAGENICITY OF 2-AMINOFLUORENE WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 12)

2026049009

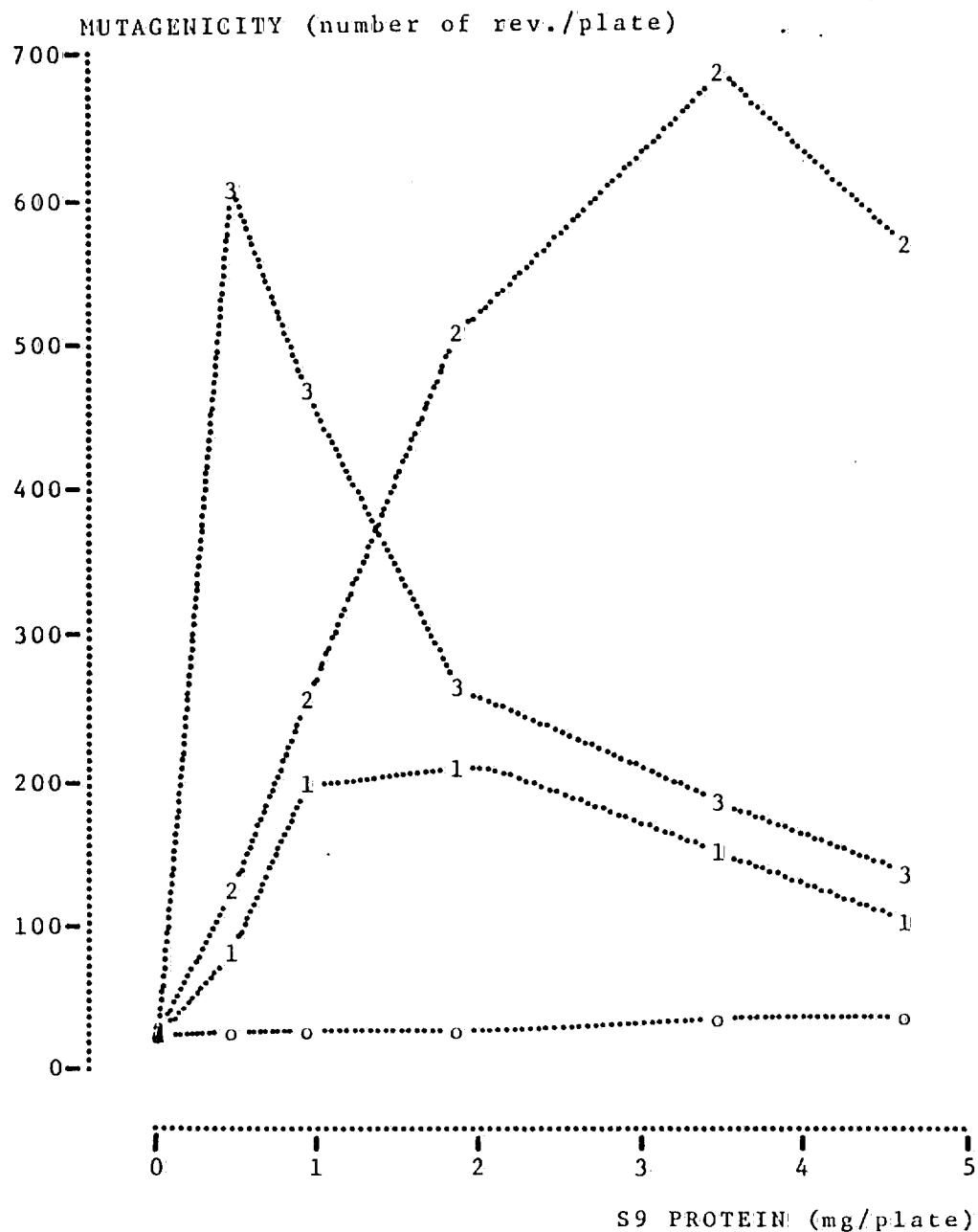


FIGURE 5

PROMUTAGEN ACTIVATION BY VARIOUS DOSES OF S9 PROTEIN,
BATCH NO. 81.A WITH STRAIN TA 98
(see TABLE 14)

- 0: DMSO, 40 μ l/plate (spontaneous reversion)
- 1: WSC-I of 2R1, 0.10 mg dry condensate/plate
- 2: B(a)P, 5 μ g/plate
- 3: 2-AF, 2 μ g/plate

date of determination: 2.Sep.81

Source: <https://www.industrydocuments.ucsf.edu/docs/gsmm0000>

2026043010

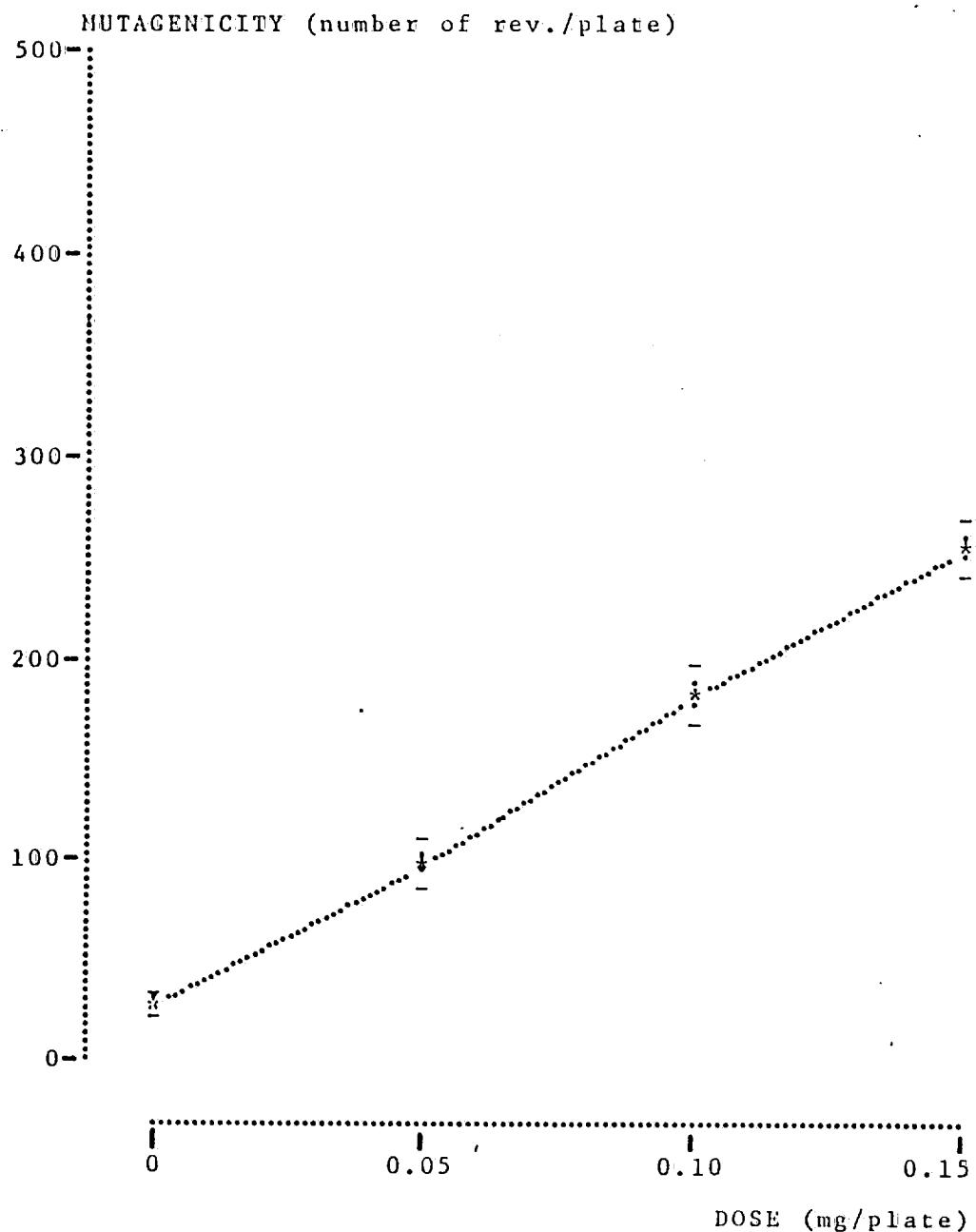


FIGURE 6

MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 16)

2026049011

A-/2059.00

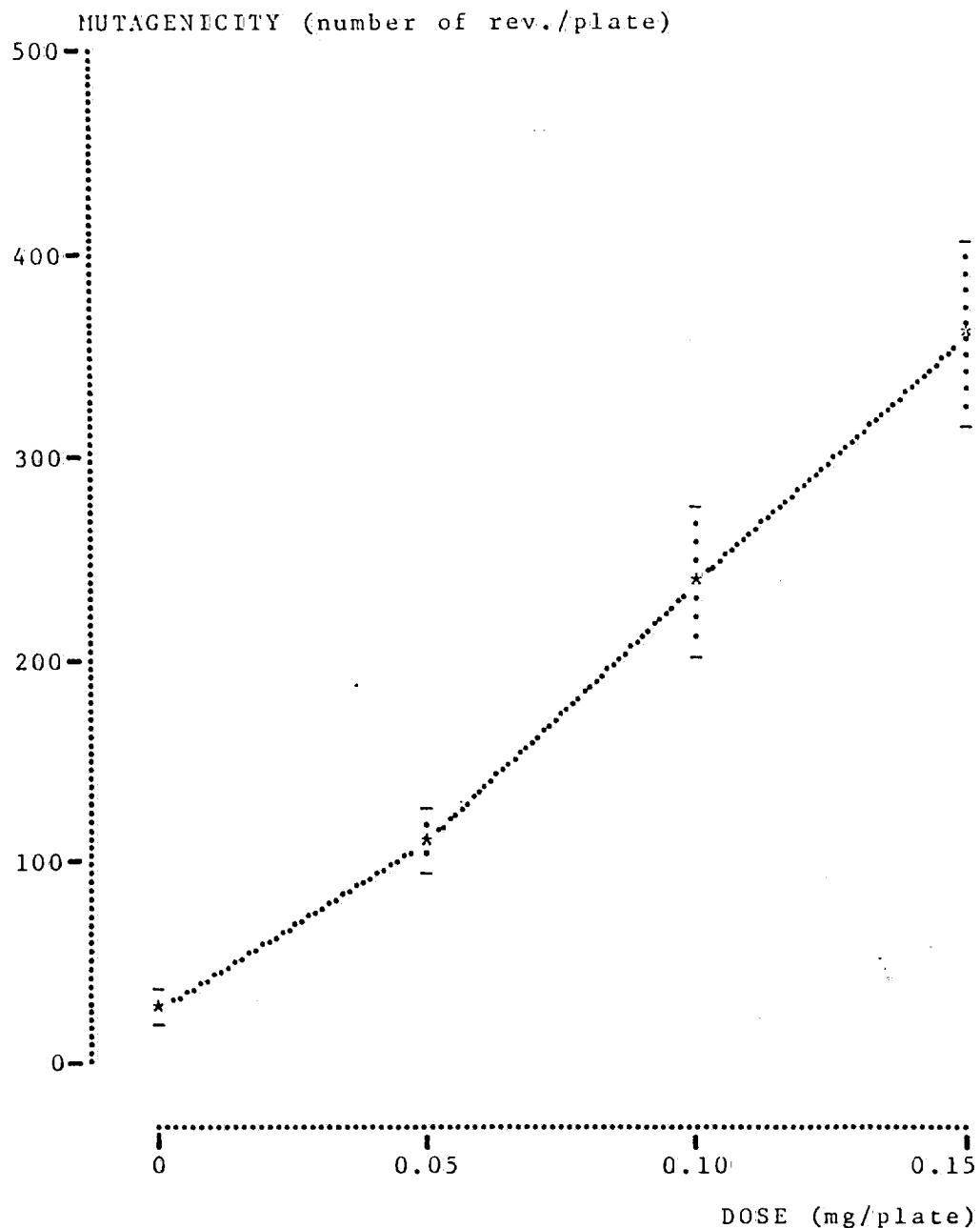


FIGURE 7

MUTAGENICITY OF WSC-I OF CIGARETTE LEAF 0-17-2
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 17)

2026049012

A-/2059.00

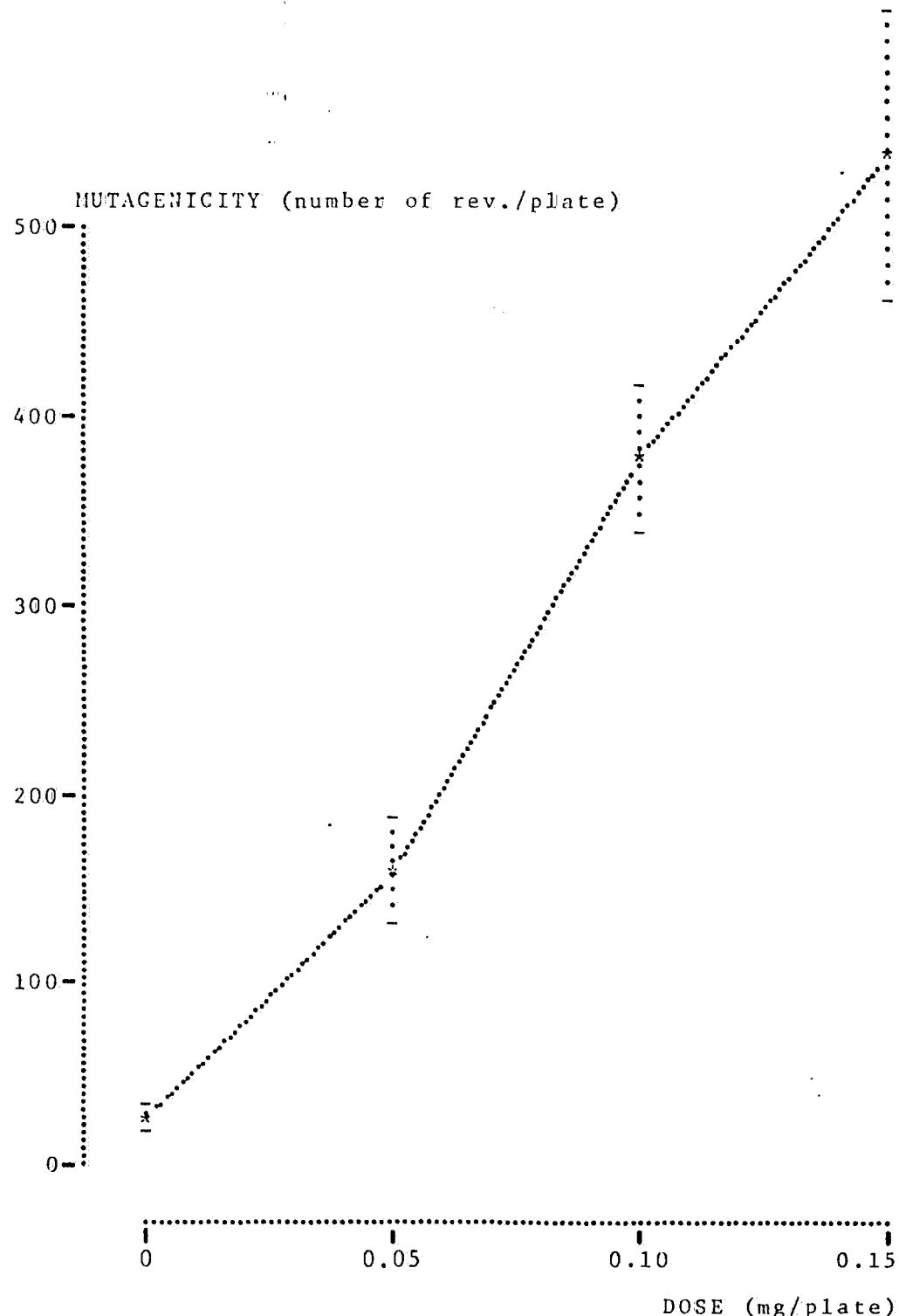


FIGURE 8

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-3
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 18)

2026049013

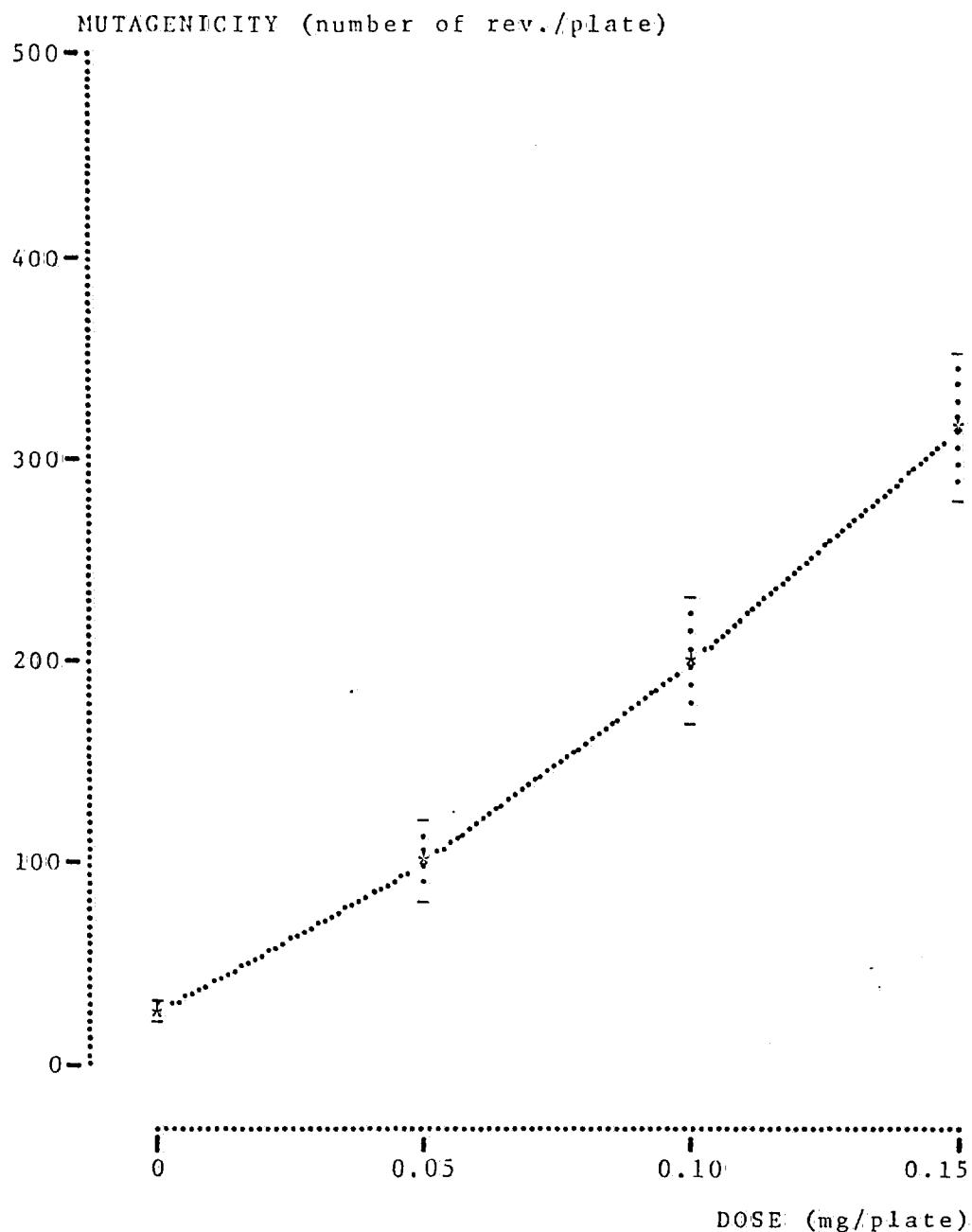


FIGURE 9

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 19)

2026049014

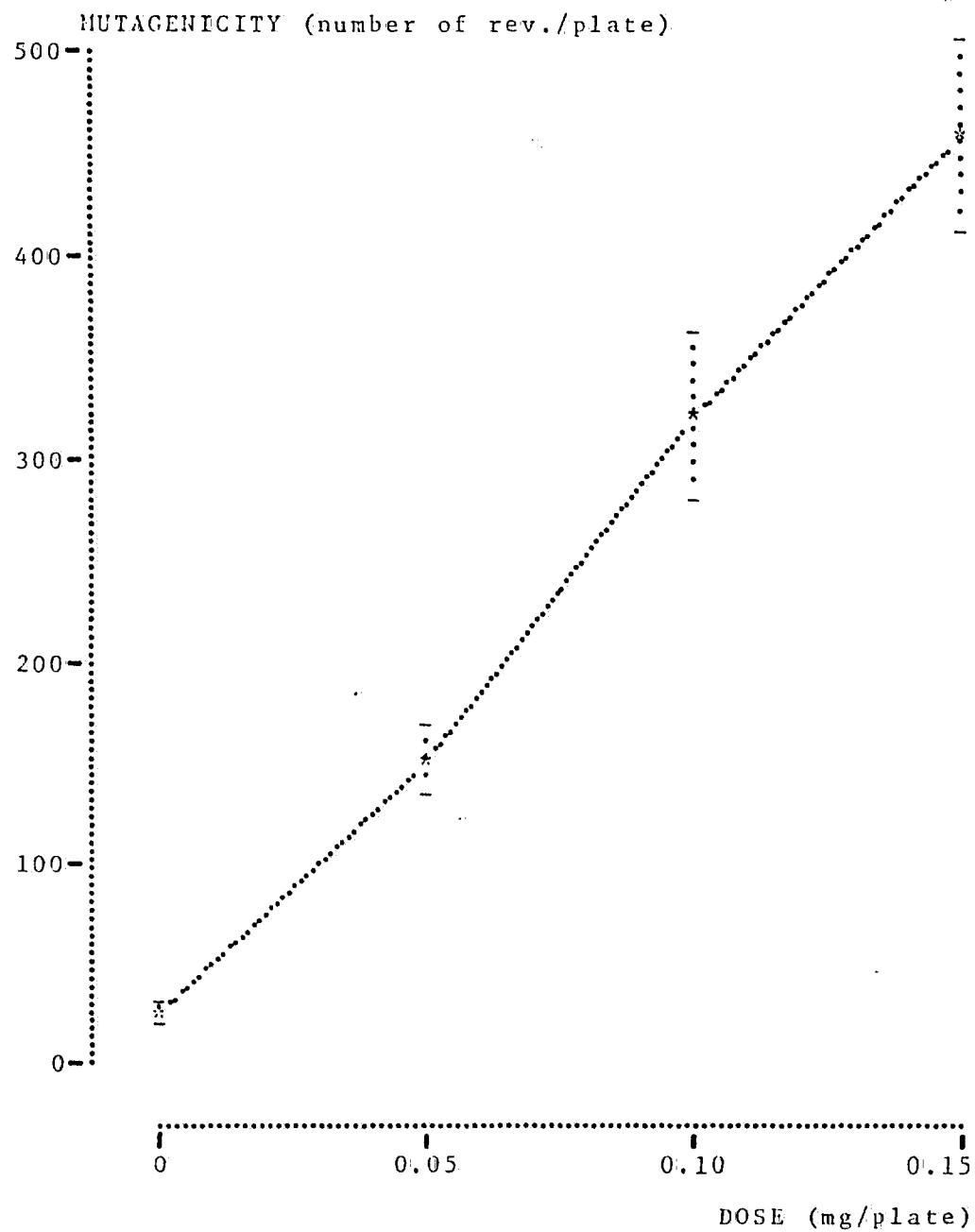


FIGURE 10

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 20)

2026049015

A-/2059.00

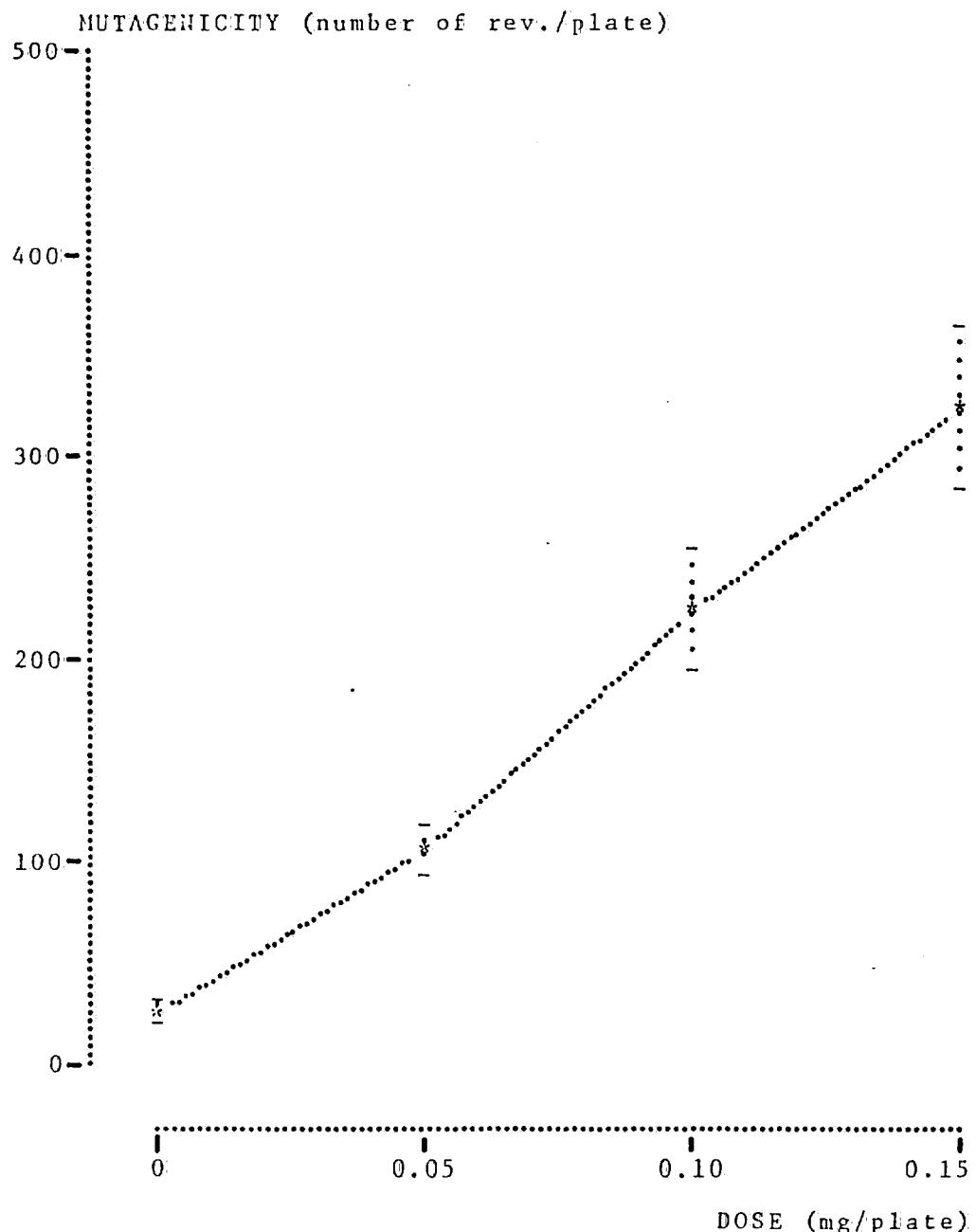


FIGURE 11

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-2
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 21)

2026043016

A-/2059.00

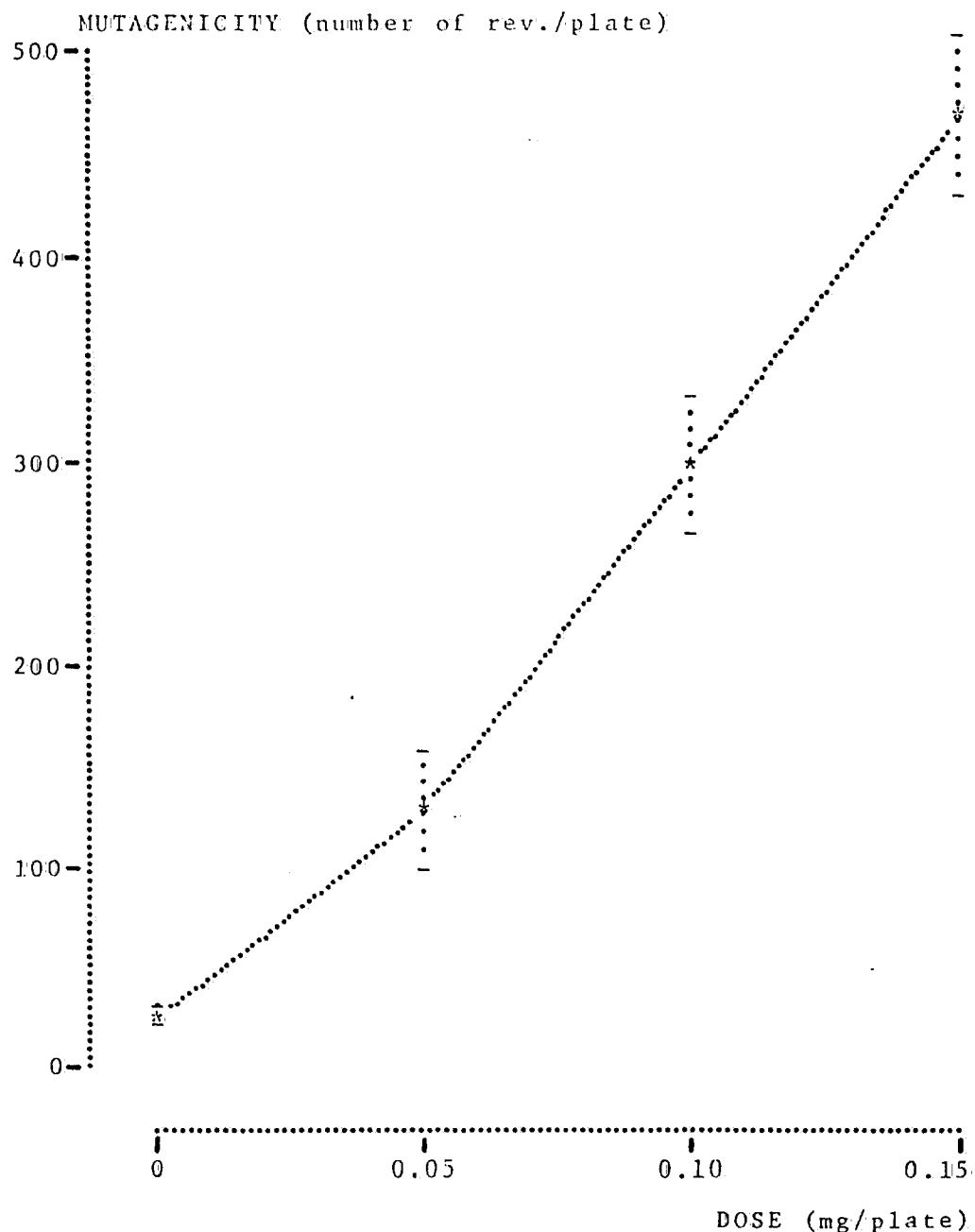


FIGURE 12

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 4-17-3
WITH S9 ACTIVATION, STRAIN TA 098
(see TABLE 22)

2026043017

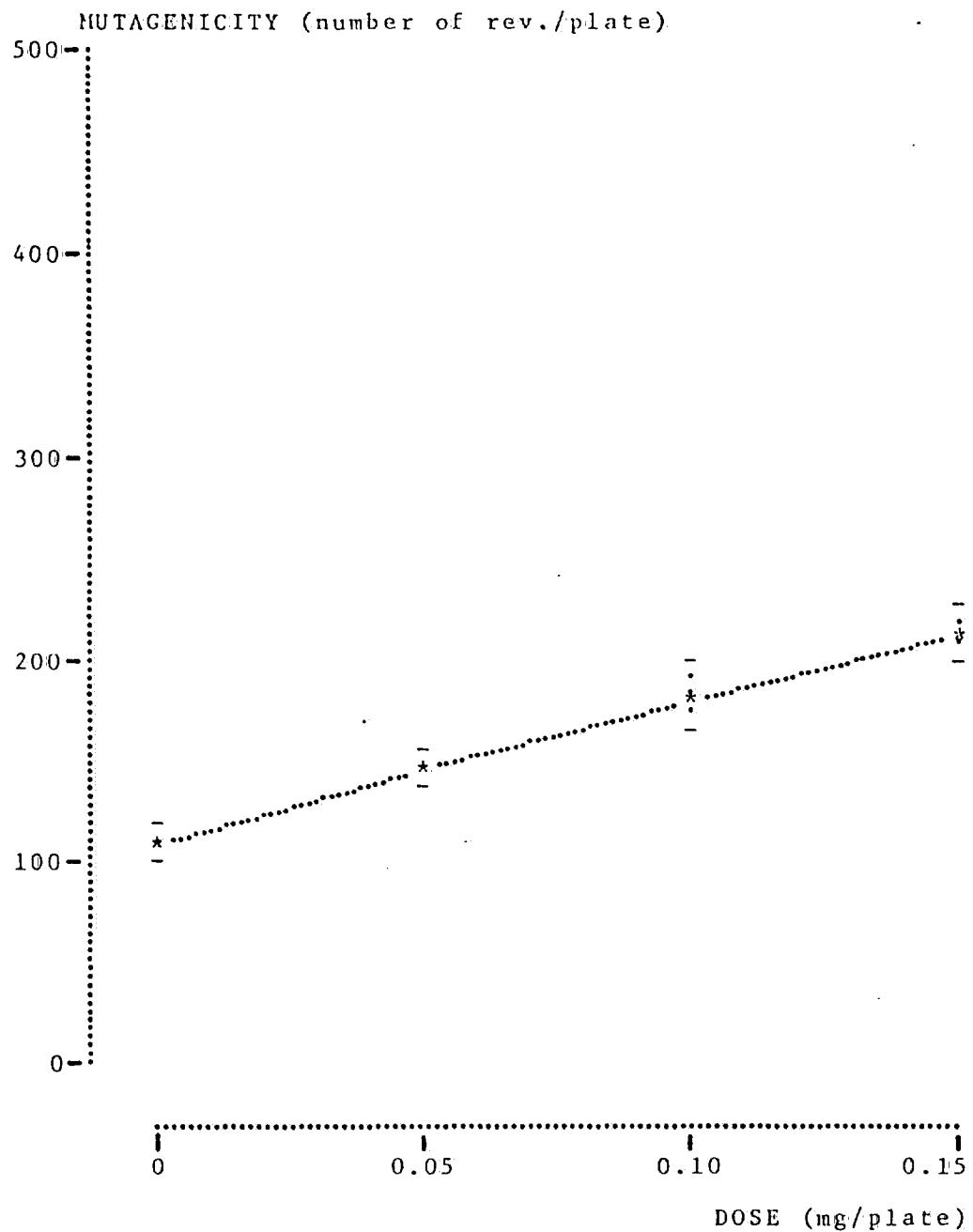


FIGURE 13

MUTAGENICITY OF WSC-I OF CIGARETTE 2R1
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 23)

2026049018

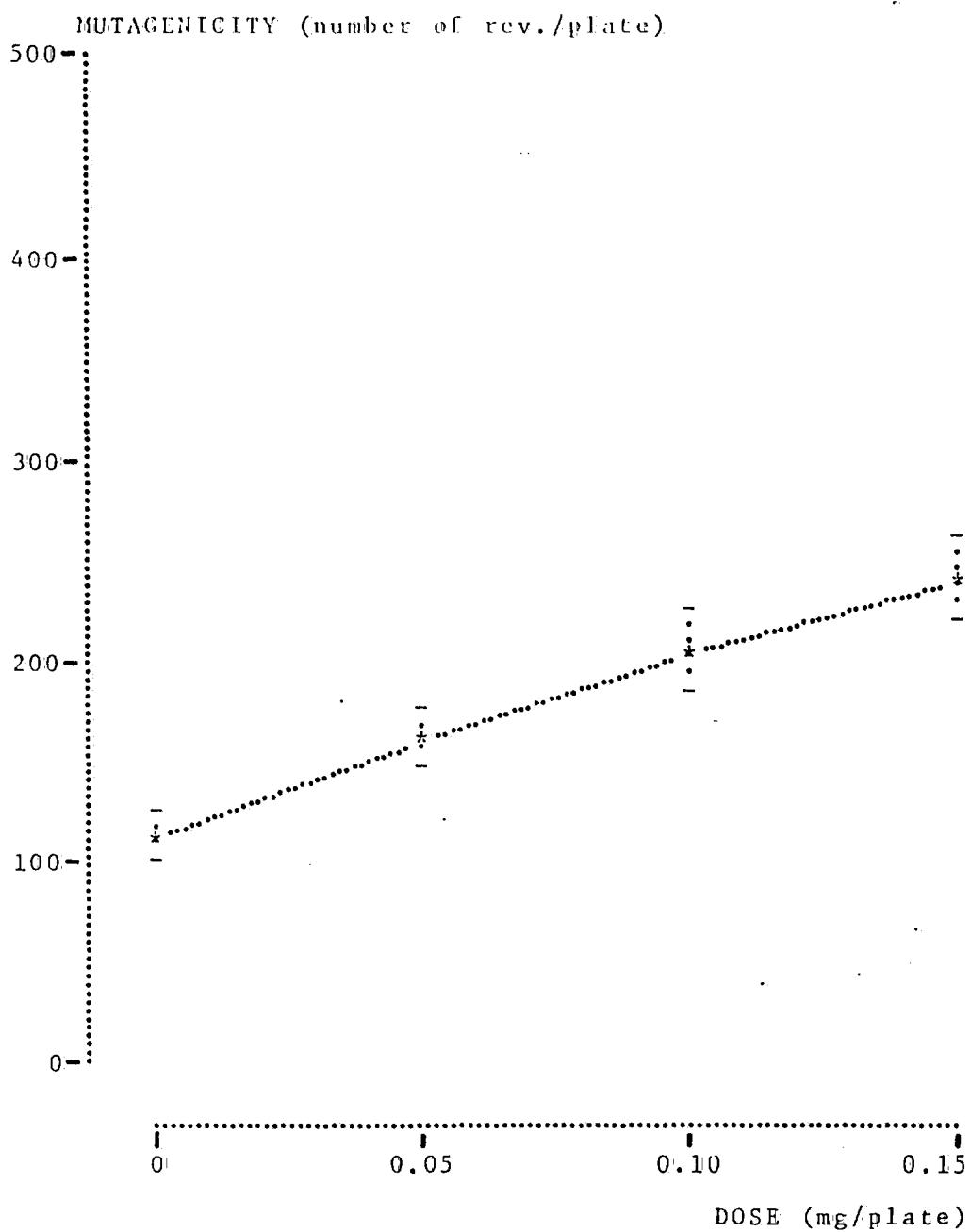


FIGURE 14

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 0-17-2
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 24)

2026049019

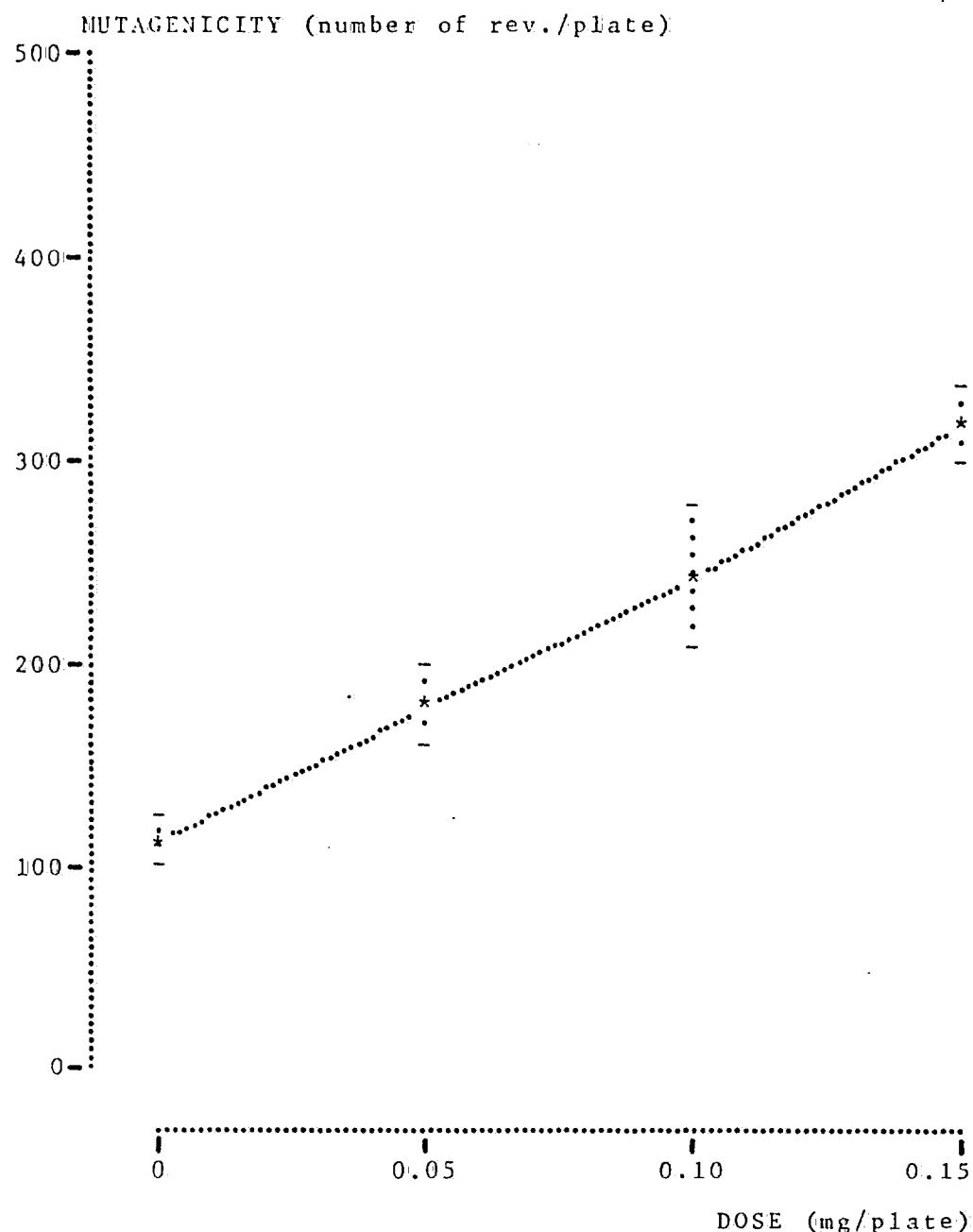


FIGURE 15

MUTAGENICITY OF WSC-I OF CIGARETTE LEAF 0-17-3
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 25)

2026043020

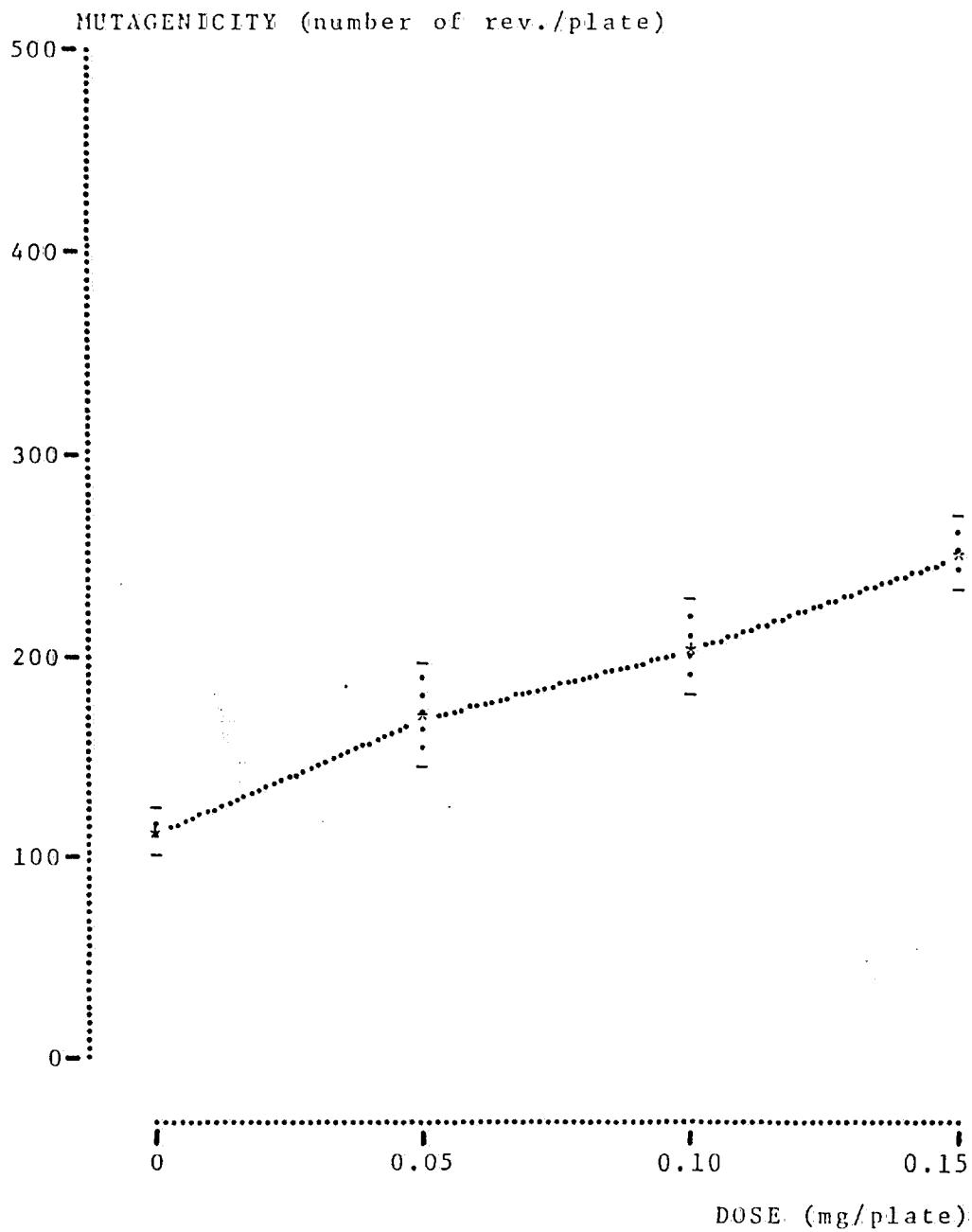


FIGURE 16

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-2
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 26)

2026043021

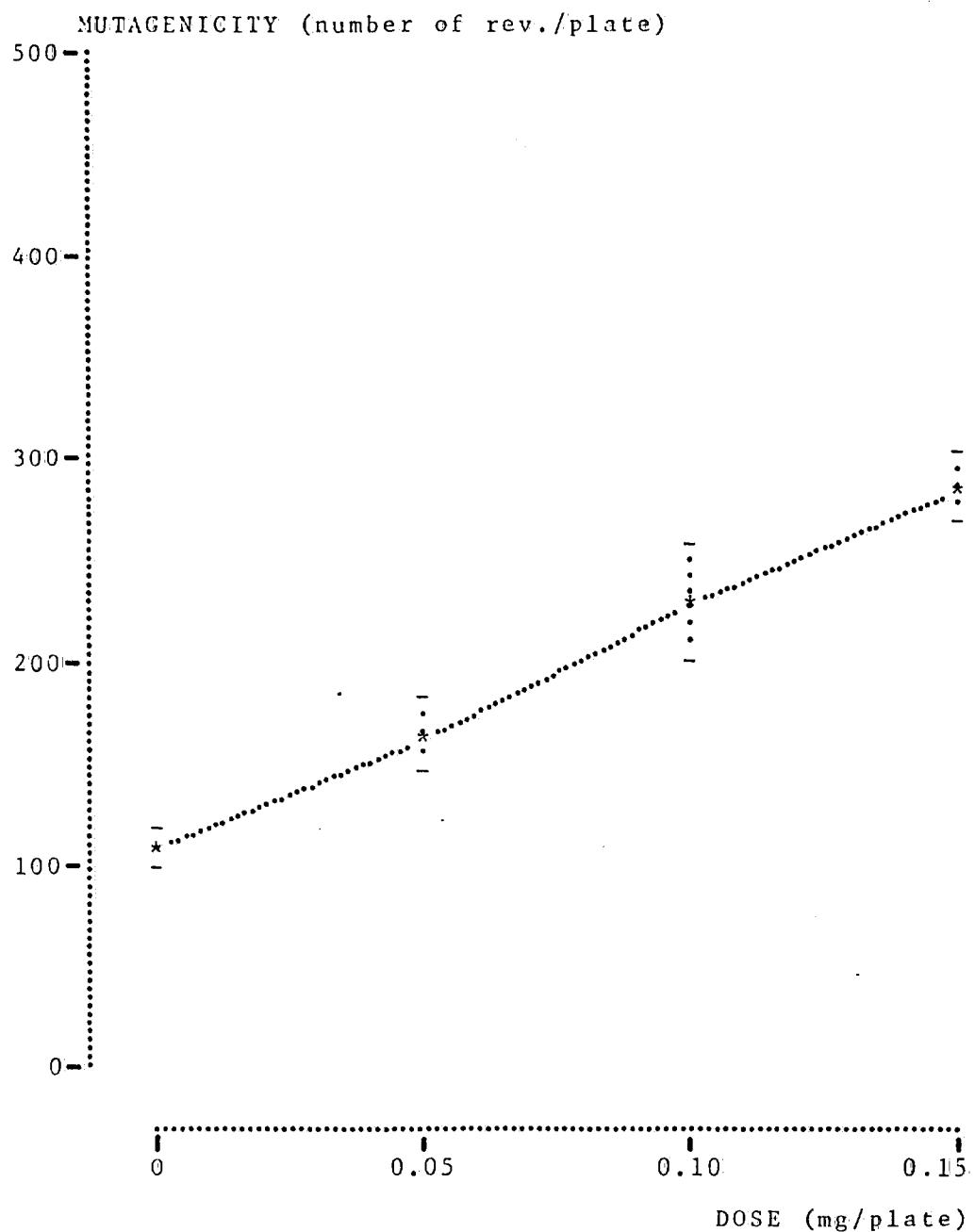


FIGURE 17

MUTAGENICITY OF WSC-I OF CIGARETTE LEAR 2-17-3
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 27.)

A-/2059.00

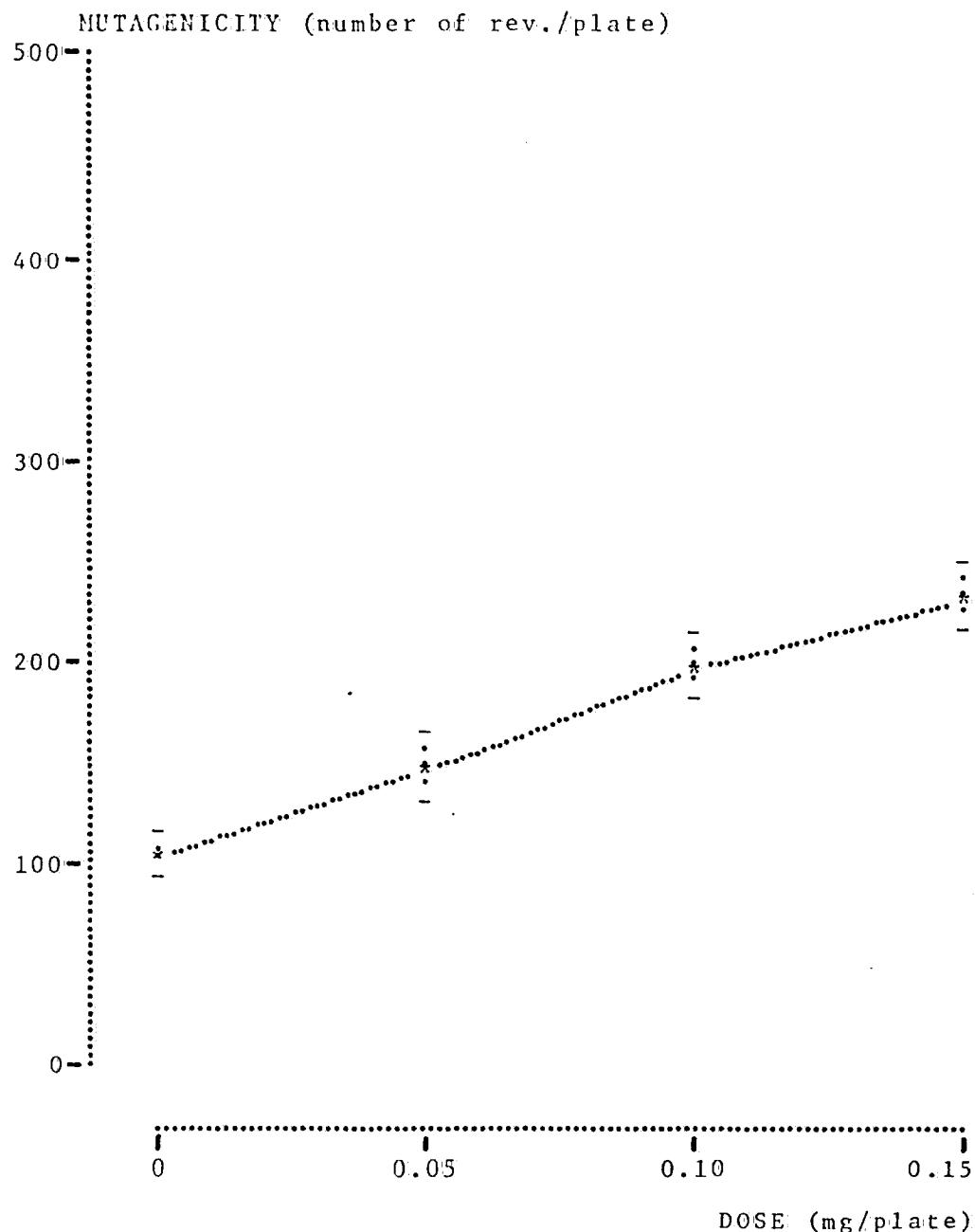


FIGURE 18

MUTAGENICITY OF WSC-I OF CIGARETTE LEAF 4-17-2
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 28)

2026049023

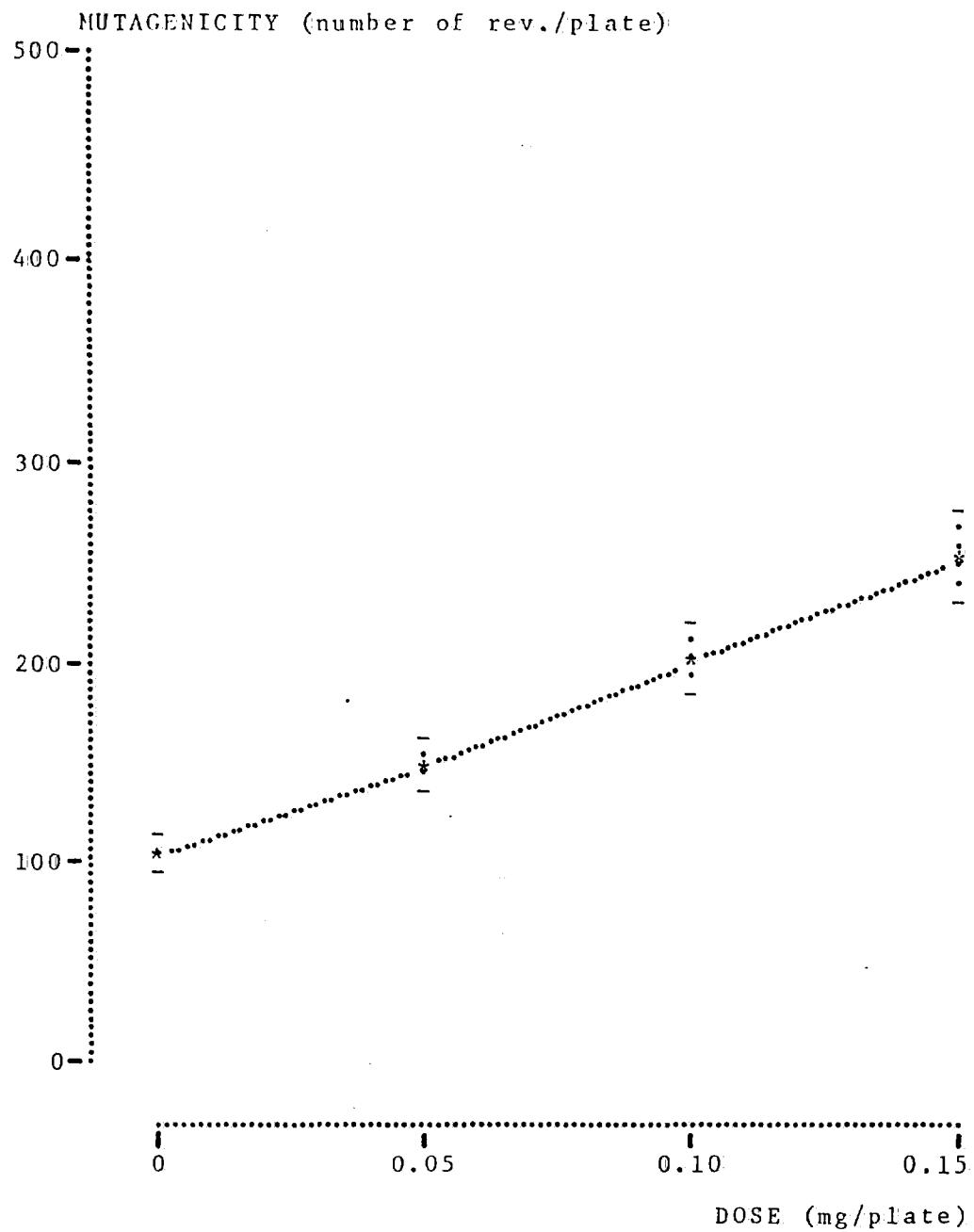


FIGURE 19

MUTAGENICITY OF WSC-I OF CIGARETTE LEAF 4-17-3
WITH S9 ACTIVATION, STRAIN TA 100
(see TABLE 29)

2026049024

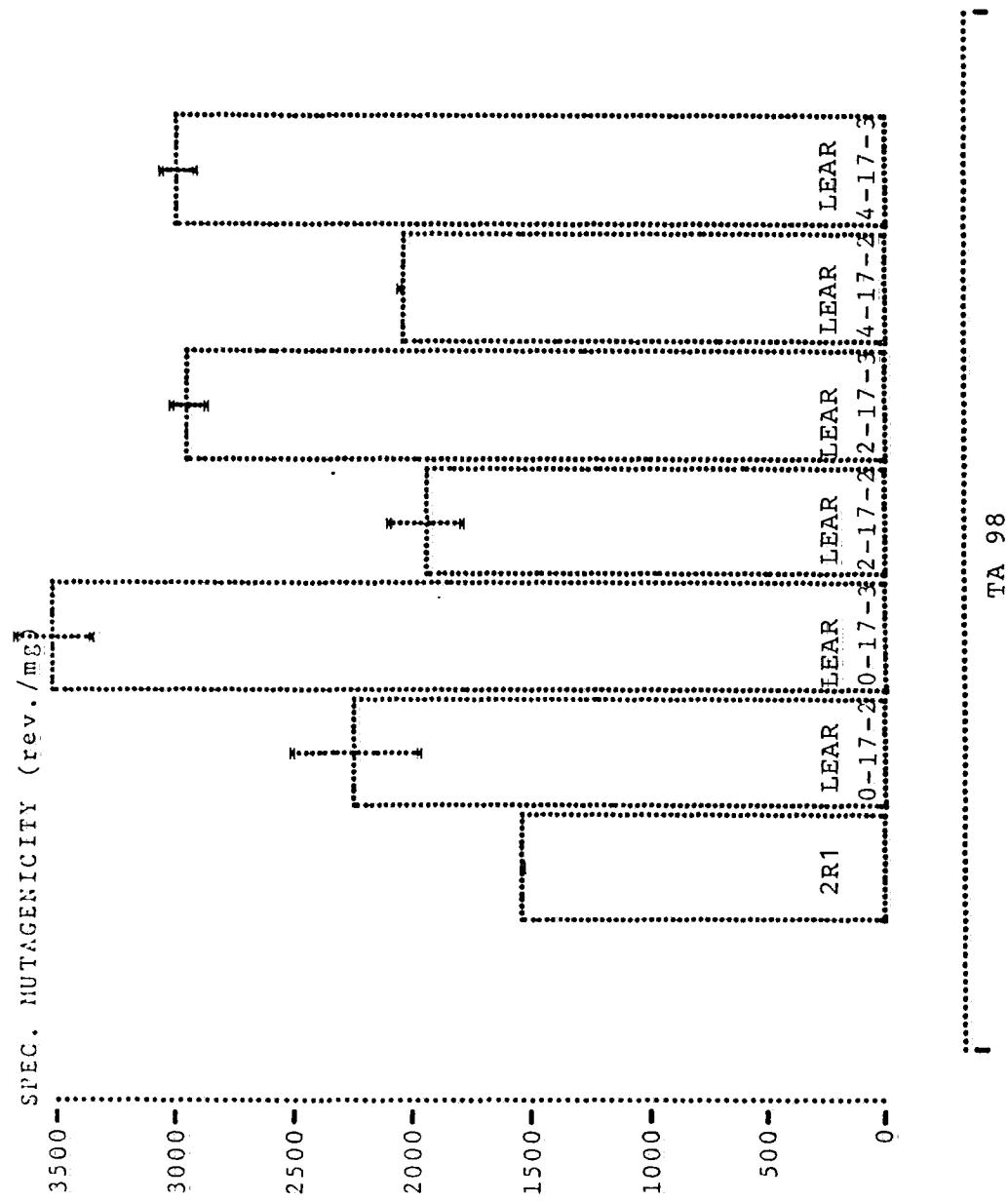


FIGURE 2o

SPECIFIC MUTAGENICITY OF CIGARETTES, STRAIN TA 98
means of assay 1 and 2 with 64 plates
(see TABLES 32 to 38)

2026049025

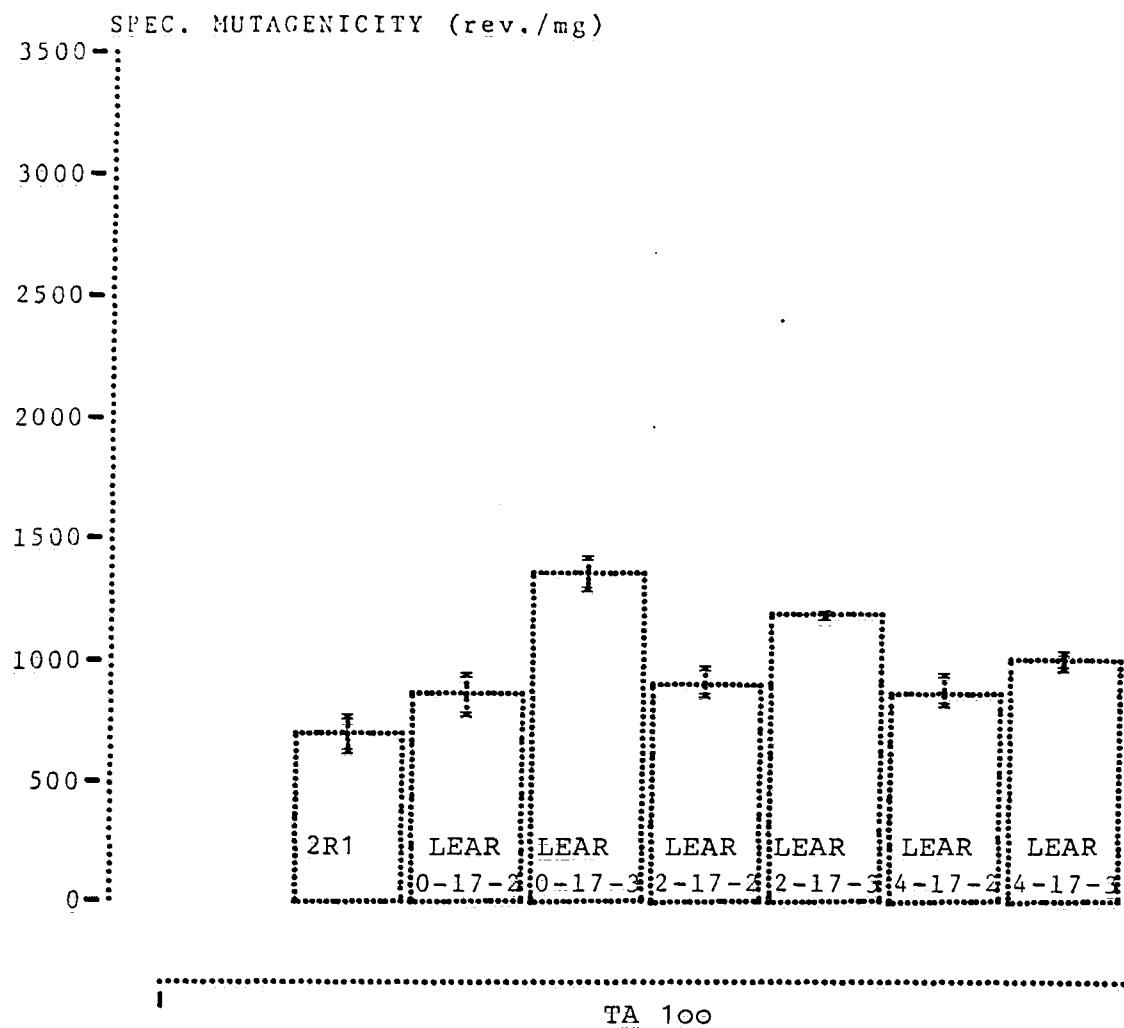


FIGURE 21

SPECIFIC MUTAGENICITY OF CIGARETTES, STRAIN TA 100

means of assay 1 and 2 with 64 plates
(see TABLES 39 to 45)

2026049026

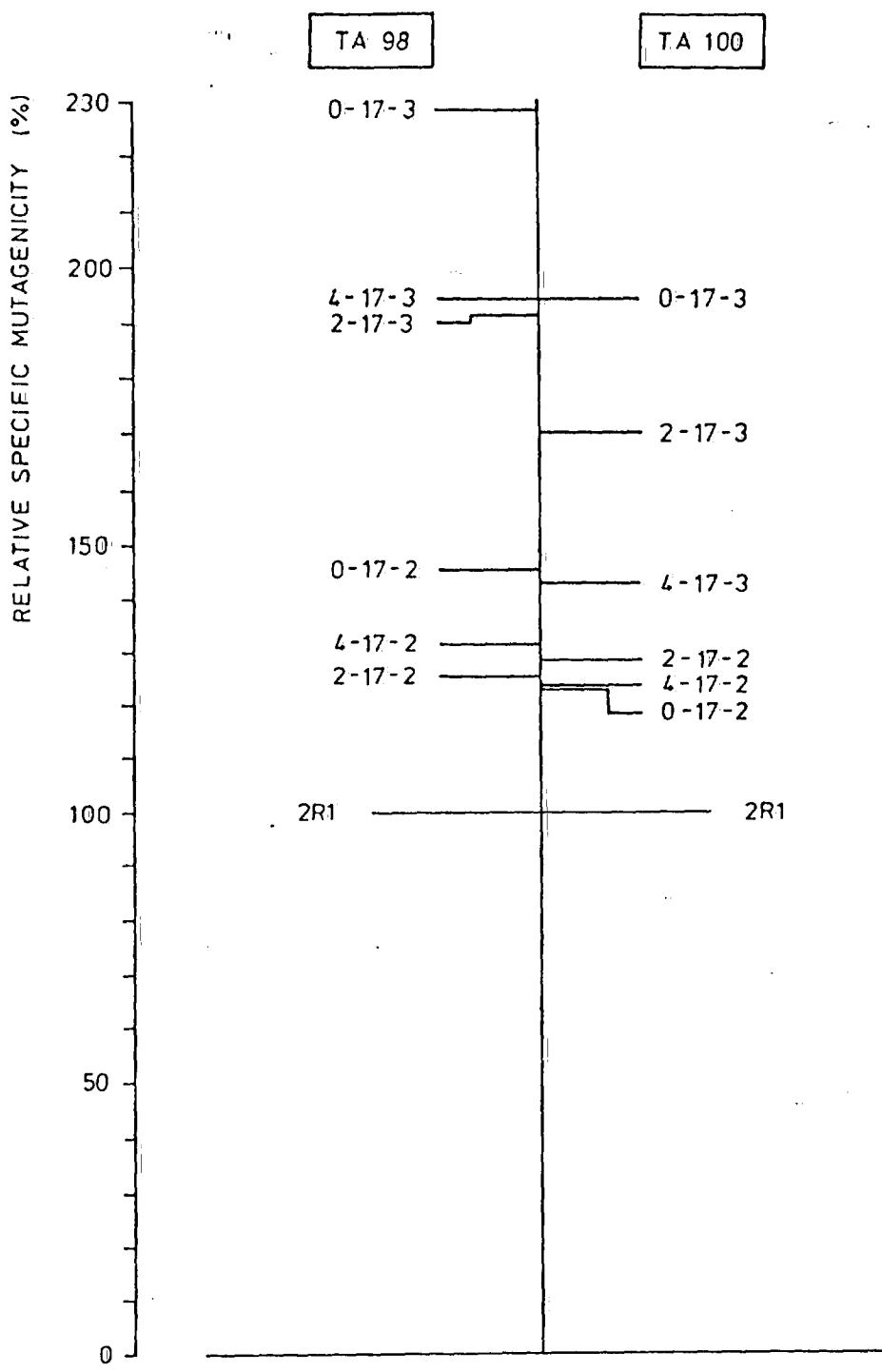


FIGURE 22

SPECIFIC MUTAGENICITY OF WSC-I OF "LEAR" CIGARETTES
RELATIVE TO WSC-I OF STANDARD REFERENCE CIGARETTES 2R1,
STRAINS TA 98 AND TA 100
(see TABLES 47 and 48)

2026049027

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END OF REPORT

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